

A Study of the Contractile Process in Frog and Mouse Skeletal Muscle and in ABRM of *Mytilus edulis*

With Special Reference to the Depressant
Effect of Active Shortening

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Malin Ekblom



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The dissertation is based on the following papers which in the text will be referred to by their Roman numerals.

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Shortening induced deactivation of skinned fibres of frog and mouse striated muscle.
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- II Ekelund, M.C. 1983.
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INTRODUCTION

It is now generally accepted that muscle contraction is based on interaction between the two contractile proteins actin and myosin. During relaxation of vertebrate striated muscle, the actin-myosin interaction is inhibited by the actin-linked troponin-tropomyosin regulatory complex (for review see Sandow 1970, Weber & Murray 1973, Ebashi 1976, Perry 1979). In molluscan muscles, this inhibition is produced by the myosin and its associated light chains (Kendrick-Jones et al. 1970, Szent-Györgyi et al. 1973, Lehman & Szent-Györgyi 1975, for review see Kendrick-Jones & Scholey 1981). An increase in the amount of activator-calcium in the myofibrillar space relieves this inhibitory effect and initiates contractile activity. The precise details in the molecular mechanisms of the actin-myosin interaction are still incompletely elucidated.

The main object of the present study has been to obtain more information concerning the basic mechanisms that are involved in the contractile process. This investigation is focused on one aspect of this problem; the effect of active shortening on the contractile behaviour.

Several previous observations have demonstrated that shortening during activity has a depressant effect on the contractile response during the remainder of the activity period. This phenomenon has been observed in both cardiac and skeletal muscle from Amphibia as well as Mammalia (Jewell & Wilkie 1960, Brady 1966, Joyce & Rack 1969, Joyce et al. 1969, Edman & Kiessling 1971, Edman & Nilsson 1971, Bozler 1972, Briden & Alpert 1972, Kaufmann et al. 1972, Dickinson & Woledge 1973, Bodem & Sonnenblick 1974). The depressant effect of active shortening has been shown in whole muscle in situ in the body (Joyce & Rack 1969, Joyce et al. 1969), in isolated whole muscle (Jewell & Wilkie 1960, Briden & Alpert 1972) and in single muscle fibres (Edman & Kiessling 1971, Edman 1975, 1980). Force depression in response to active shortening thus appears to be a feature of striated muscle in general. In spite of this, only certain characteristics of the shortening effect in vertebrate skeletal muscle have been described (Edman 1975, 1980).

An essential feature of the shortening induced depression is that it disappears completely during continued stimulation of the muscle, i.e.

it represents a reversible change within the contractile system. Furthermore, the magnitude of the shortening effect was found to be related to the extent of shortening while the load and velocity of shortening had no significant influence (Edman 1975).

As regards the mechanism underlying the depressant effect of active shortening, previous observations on intact single muscle fibres give certain clues. The shortening effect did not influence V_0 , the measured velocity of shortening at zero load. This observation can be presumed to exclude that the shortening induced depression is due to an increased passive resistance to filament sliding. The relationship between force and velocity of shortening at loads close to zero is very steep. An increased 'intrinsic load' would therefore cause a reduction of V_0 . The gradual disappearance of the shortening effect during continued activation of the preparation would seem to rule out sarcomere non-uniformity as the cause of force depression. The effect of a non-uniform distribution of sarcomere length would be expected to persist or become even more augmented during continued contractile activity. Furthermore, the reversibility of the shortening effect during continued isometric contraction as well as its independence, over a wide range, of the load and work output during the shortening phase suggests that the force depression by shortening is not attributable to depletion of chemical energy in the contractile process. Studies of intact single frog muscle fibres gave no evidence of altered kinetics of activator-calcium in the excitation-contraction coupling in response to active shortening. The shortening effect was long-lasting, did not disappear merely by restimulation of the fibre and did not essentially alter the relative time-course of the mechanical response. These qualities indicate that neither the release nor the re-uptake of activator-calcium in the excitation-contraction process is essentially affected by active shortening (Edman 1975, 1980). The proposal that active shortening exerts a depressant effect on the myofilament system per se would seem to remain as the most likely explanation (Edman 1975, 1980). The low temperature dependence of the shortening induced depression and the lifetime of the depressant effect, approximately 1 s, were interpreted as evidence in favour of this opinion (Edman 1975, 1980).

It was proposed that the depressant effect of active shortening is due to a reduced affinity of the regulatory troponin-tropomyosin system for activator-calcium (Edman 1980). The aim of the present study has been to

explore this possibility more thoroughly.

A reduction in affinity of the actin-linked regulatory complex for calcium can be assumed to exhibit a relationship to the amount of activator-calcium in the myofibrillar space. Papers I and III elucidate this connection from various points of view. Paper III describes the depressant effect of active shortening in intact single muscle fibres of the frog during single twitch and fused tetanic contraction. The influence of substances which potentiate or reduce the isometric force production is studied. Diazepam, pentobarbital and 4-aminopyridine all potentiate the isometric twitch contraction, by virtue of altering the kinetics of activator-calcium in the excitation-contraction coupling (e.g. Khan & Edman 1979, 1983 In press, DeGroof et al. 1980, Khan 1980). Conversely, dantrolene reduces the isometric tension, presumably by decreasing calcium release during membrane excitation (van Winkle 1976, Desmedt & Hainaut 1977, Morgan & Bryant 1977). Paper I describes the effect of the free calcium concentration on the shortening induced depression in chemically skinned fibre segments from both amphibian and mammalian striated muscle. In this kind of preparation the concentration of various ions and ion complexes in the medium surrounding the myofilaments can be controlled.

The binding of calcium to troponin is likely to be modulated during the course of contraction. A local feedback control is thought to be exerted by the myosin cross-bridges (Adelstein & Eisenberg 1980, cf. also Bremel & Weber 1972, Weber & Murray 1973). It follows from this that alteration in the actin-myosin interaction, as during active shortening, would have the ability to indirectly affect the affinity of troponin for activator-calcium. Variations in the rate of dissociation of cross-bridges would for this reason be expected to modulate the magnitude of the depressant effect of active shortening. Several observations have shown that an increase of the ionic strength enhances the speed of dissociation of heavy meromyosin from actin (Eisenberg & Moos 1970, Rizzino et al. 1970, Greene 1981). The influence of direct variations in ionic strength on the shortening induced depression in chemically skinned fibres is described in paper I. In the intact isolated fibre, varied tonicity of the extracellular medium alters the intracellular ionic strength (paper II). This part of the study also tried to evaluate the effect of tonicity induced variations in myofilament lattice width on the depressant effect of active shortening.

The binding of activator-calcium to troponin can be presumed to be modulated by changes of the conformational state of the troponin molecule. It has been proposed that binding of magnesium to the Ca^{2+} - Mg^{2+} sites on the contractile proteins is of relevance for the conformation of troponin (Levine et al. 1978, Potter et al. 1981, cf. also Miki et al. 1982, Zot & Potter 1982). The influence of variations in the magnesium concentration on the shortening effect was studied in skinned fibre segments (paper I).

It was of interest to investigate the effect of active shortening in a muscle that is considered to lack the calcium-regulatory troponin-tropomyosin system. For this reason, the depressant effect of active shortening in the anterior byssus retractor muscle (ABRM) of the mollusc Mytilus edulis was studied (paper IV). In this muscle, the calcium-regulatory mechanism is considered to be essentially located to the myosin and its associated light chains (for references see above).

METHODS

Preparations and mounting.

A. Single muscle fibres were dissected from the semitendinosus and tibialis anterior muscles of Rana temporaria.

B. In one series of experiments, the anterior byssus retractor muscle (ABRM) of the mollusc Mytilus edulis was studied.

C. Chemically skinned fibre segments were obtained from the semitendinosus muscle of Rana temporaria and the psoas muscle of the mouse.

The preparation was mounted horizontally in a thermostatically controlled chamber between hooks of steel wire extending from a force transducer and an electromagnetic puller, respectively.

The muscle bath was surrounded by a jacket through which a thermostatically controlled water-glycol solution from a Colara Ultrathermostat was circulated. The temperature within any given experiment was thereby maintained constant to $\pm 0.3^{\circ}\text{C}$. In order to allow passage of a laser beam (see further below), the floor of the chamber was made of a microscope slide.

Solutions.

The compositions of the different solutions used are given in papers I-IV.

Stimulation.

A. The intact single muscle fibres were stimulated to contract by passing current through two platinum plate electrodes placed on either side of the fibre. Rectangular pulses of 0.2 ms duration were used and the stimulus strength applied was approximately 25% above threshold.

B. ABRM was stimulated by alternating current (50 Hz) via plate electrodes placed along the preparation. The field strength was 1.7 V mm^{-1} .

C. Contractures of the chemically skinned fibre segments were induced by immersing the preparation in a Ca^{2+} -containing solution.

Determination of sarcomere spacing.

The sarcomere length at rest and during activity in the vertebrate muscle preparations was determined by using essentially the same laser diffraction technique as was described by Cleworth & Edman (1972). Changes in sarcomere length after releases of various amplitude were calculated from the change in overall fibre length and the sarcomere spacing at the onset of the lever movement.

Electromagnetic puller.

Controlled length changes of the preparation were produced by means of an electromagnetic puller. The speed of the release steps was sufficiently high to prevent force production during the shortening phase.

Tension recording.

For recording of tension, a semiconductor strain-gauge transducer (AE 801 Aksjeselskapet Mikroelektronikk) was utilized. This type of transducer has previously been described by Edman (1980).

Recording and measurement of responses.

Tension recordings and release steps were displayed on a Tektronix 5103N or 5113N storage oscilloscope and were photographed on 35 mm orthochromatic film. Measurements from the film records were performed in a Nikon model 6C profile projector at 10 x magnification using the stage micrometer readings.

For determinations of statistical significance, Student's t-test was utilized.

More detailed descriptions concerning the experimental techniques used are given in papers I-IV.

RESULTS & DISCUSSION

A. Determination of the depressant effect of active shortening

1. Single muscle fibres of frog and anterior byssus retractor muscle (ABRM) of *Mytilus edulis*.

A small control step was applied during the rising phase of a twitch contraction or prior to the last stimulus during a fused tetanus in vertebrate skeletal muscle (e.g. Fig. 1, paper III) and during the rising phase of a phasic contraction in ABRM (Fig. 1, paper IV), respectively. This step was just large enough to cause a complete drop in tension followed by immediate redevelopment of force. A larger test release which allowed shortening over an additional distance was applied and was carefully timed so that force redevelopment after this step was initiated at the same moment during the contraction as after the control release. Force redevelopment after both control and test releases occurred at muscle lengths essentially within the plateau of the length-tension relation for the muscle studied (e.g. Gordon et al. 1966, Edman 1966, Cornelius & Lowy 1978). The magnitude of the depressant effect of active shortening was measured as the difference in peak redeveloped force after the control and test releases. This difference was related to the force redeveloped after the small control release step or, in certain experiments, to the unreleased isometric contractile response.

2. Chemically skinned fibre segments of vertebrate striated muscle.

In this series of experiments, the control step was applied when almost optimal isometric tension had been obtained in the calcium-containing 'contracture' solution. The test release was initiated during the same contraction, after a fixed time, when full contractile force had been restored (Fig. 2, paper I). Alternatively, the reversed order of the two release steps was used. The shortening induced depression was calculated as the difference in rate of force redevelopment, at a given force level, after the control and test steps. This difference was expressed in per cent of the value measured after the control release (Fig. 5, inset, paper I).

B. The depressant effect of active shortening in chemically skinned fibres of frog and mouse striated muscle (paper I)

A depressant effect in response to active shortening, very similar to that observed in the intact system (Edman 1980), was obtained in chemically skinned fibres of the frog (Fig. 2). Furthermore, the same finding was made in membrane-free preparations of mammalian striated muscle (Fig. 3). These observations exclude the possibility that the shortening induced depression results from altered kinetics of release and re-uptake of activator-calcium in the excitation-contraction coupling.

In conformity with previous observations obtained in intact skeletal muscle fibres (Edman 1975), the shortening effect was entirely reversible and disappeared completely during continued contractile activity. This feature suggests that the depressant effect of active shortening is not due to non-uniformity in sarcomere length. In that case, the shortening induced depression would be expected to remain or to augment during continued isometric activity. The observed critical dependence of the shortening effect upon the ionic milieu of the surrounding medium provides additional evidence in favour of the view that the effect reflects an alteration in the contractile system itself (see further above).

An increase of the free calcium concentration (from 1.5 μM to 12.0 μM) around the myofilaments resulted in a progressive reduction of the shortening induced depression in both the frog and the mouse fibre preparations (Fig. 5 and Fig. 6, respectively). The finding that the depressant effect of shortening is counteracted by increased concentration of Ca^{2+} supports the view that active shortening leads to decreased affinity of troponin for activator-calcium. An appropriate increase of the calcium ion concentration would be expected to entirely eliminate the shortening effect (cf. paper III). This prediction was difficult to test as the chemically skinned preparations exhibited signs of damage at very high calcium ion concentrations ($> 12 \mu\text{M}$). However, raising the calcium ion concentration to 6-12 μM was sufficient to almost completely neutralize the depressant effect of shortening in some of the fibres.

A reduction of the shortening induced depression was observed as the ionic strength was increased (Fig. 8). An enhancement of the ionic strength from 105 mM to 235 mM resulted in a reduction of the shortening effect that amounted to approximately 40%. Biochemical data suggest that the cross-bridges exert a feedback control on the calcium binding to the regulatory system. This feature has been included in the kinetic model pre-

sented by Adelstein & Eisenberg (1980). Increased ionic strength enhances the speed of dissociation of heavy meromyosin from actin (Eisenberg & Moos 1970, Rizzino et al. 1970, Greene 1981). It is possible that this effect of ionic strength on the dissociation rate of the cross-bridges may indirectly affect the troponin-calcium binding equilibrium and thereby the state of activity of the contractile system.

The depressant effect in response to a given amount of shortening was found to be approximately doubled as the magnesium ion concentration increased from 25 μM to 590 μM (Fig. 7). This observation may likewise reflect a feedback influence of the myosin cross-bridges. It has been proposed that magnesium bound to the $\text{Ca}^{2+}\text{-Mg}^{2+}$ sites on the regulatory proteins modulates the conformation of troponin (Levine et al. 1978, Potter et al. 1981). It is conceivable that increased binding of magnesium to troponin enhances the feedback control of the cross-bridges and in this way augments the depressant effect of active shortening.

C. The shortening induced depression in intact single muscle fibres of the frog at varied tonicity of the extracellular medium (paper II)

The depressant effect of active shortening in isolated skeletal muscle fibres was found to be inversely related to the tonicity of the extracellular medium (Table 2 and Table 3). The solution was made hypotonic by reducing the NaCl concentration from 115.5 mM to 92.4 mM and 69.3 mM. These alterations resulted in a relative tonicity of 0.81T and 0.62T, respectively, of the normotonic solution (1.00T). Hypertonic solutions were prepared by addition of 50.0 mM and 98.0 mM sucrose to the normotonic solution. The relative tonicity thereby amounted to 1.22T and 1.44T, respectively.

Within the tonicity range used in this study (0.62T - 1.44T), the observed effects on the shortening induced depression were most likely attributable to altered intracellular ionic strength and/or a change in myofilament lattice width.

The muscle fibre is known to maintain an almost constant volume as its length is changed (e.g. Elliott et al. 1963, Elliott et al. 1967, Huxley 1969). This feature offered a possibility to examine whether the effect of varied tonicity on the shortening induced depression was due to alteration in fibre width. The shortening effect in ordinary Ringer solution at 2.35 μm sarcomere length was compared to that obtained in hypertonic (1.22T) solution at a sarcomere length of 2.14 μm . In these two situations

the myofilament lattice width can be estimated to be approximately the same (cf. Edman & Hwang 1977). The reduction of the shortening induced depression in the hypertonic solution remained essentially unaltered after this intervention. This finding therefore suggests that altered tonicity modulates the depressant effect of active shortening by virtue of changing the intracellular ionic strength. That a change in ionic strength does affect the shortening induced depression was demonstrated in a more direct way on the skinned fibre preparation (paper I).

It has been demonstrated that the rate of dissociation of heavy mero-myosin from actin is related to the ionic strength (Eisenberg & Moos 1970, Rizzino et al. 1970, Greene 1981). The idea has furthermore been advanced that the cross-bridges exert a feedback control on the binding of activator-calcium to the regulatory complex (Adelstein & Eisenberg 1980). It is proposed that a change in ionic strength (induced by altered tonicity in the intact fibre) may in this way affect the troponin-calcium binding and thereby modulate the magnitude of the depressant effect of active shortening (paper I).

D. The shortening effect during twitch and tetanus in isolated frog muscle fibres; the influence of dantrolene, diazepam, pentobarbital and 4-aminopyridine (paper III)

Dantrolene, diazepam, pentobarbital and 4-aminopyridine are all presumed to affect the kinetics of the release and re-uptake of activator-calcium in the excitation-contraction process (e.g. Ellis & Carpenter 1971, Ellis & Bryant 1972, Foulks et al. 1973, van Winkle 1976, Desmedt & Hainaut 1977, Morgan & Bryant 1977, Khan & Edman 1979, 1983 In press, DeGroof et al. 1980, Khan 1980). Diazepam, pentobarbital and 4-aminopyridine have a marked twitch potentiating effect which is most likely the result of an increased amount of calcium in the myofibrillar space. The concentration of each of these substances that was used in this paper is considered to produce optimal twitch enhancing effect. In the present study, addition of 1.0 mM pentobarbital, 150 μ M diazepam and 3.0 mM 4-aminopyridine resulted in a twitch potentiation that amounted to 90.7%, 94.4% and 97.5%, respectively, of the isometric tetanic force in ordinary Ringer solution (mean values, paired observations). Dantrolene is known to reduce the isometric contractile response. This is probably due to a diminished release of calcium from the sarcoplasmic reticulum in response to membrane excitation.

10 μ M dantrolene was found to reduce the isometric twitch response by approximately 97% while the tetanus was reduced by about 7% (mean values) in the present investigation.

The depressant effect of a given amount of active shortening during twitch contraction in ordinary Ringer solution decreased to approximately one tenth after addition of 150 μ M diazepam (Fig. 5). Pentobarbital (1.0 mM) was capable of reducing the shortening effect during the twitch by 95-100% (Fig. 8). 4-aminopyridine (3.0 mM) entirely eliminated the shortening induced depression during twitch contraction (Fig. 10), while the depressant effect during the tetanus was reduced to approximately half the value of that obtained after the same amount of shortening in the ordinary Ringer solution (Fig. 12). Dantrolene (10 μ M), on the other hand, approximately doubled the shortening induced depression during both twitch and tetanus (Fig. 2 and Fig. 3).

An inverse relationship between the depressant effect of active shortening and the free calcium concentration in the surrounding medium has been demonstrated in the skinned fibre preparation (paper I). This study suggests that the magnitude of the shortening effect is critically dependent on the amount of calcium in the myofibrillar space also in the intact fibre. Substances that are thought to raise the calcium concentration thus caused a marked reduction of the depressant effect of active shortening during twitch contraction. In several cases, the shortening induced depression was entirely eliminated. Conversely, an enhancement of the shortening effect was obtained by an agent that is presumed to reduce the amount of calcium around the myofilaments. All these findings are fully in line with the assumption that active shortening causes a reduction in affinity of the regulatory complex for activator-calcium.

The sensitivity to active shortening appeared to be more pronounced after steady state force production had been attained than during the development of contractile activity. Thus, addition of 4-aminopyridine entirely neutralized the depressant effect of a given amount of shortening during the twitch, while the shortening effect was reduced, but not eliminated during the tetanus (compare Fig. 10B with Fig. 12). Furthermore, the isometric force level was approximately the same during a potentiated twitch after addition of pentobarbital and during a tetanus after addition of dantrolene, respectively. However, the depressant effect after a given amount of active shortening was considerably smaller in the former situation (compare Fig. 8B with Fig. 3). These differences may be due to increa-

sed sensitivity to active shortening and a smaller capability to neutralize the shortening effect after attainment of steady state force production than during the development of contractile activity.

The shortening induced depression in ordinary Ringer solution is less pronounced during a tetanus than during a twitch. The excess of free calcium that seems to be available during tetanic contraction in normal Ringer (Edman & Flitney 1982, see also Hellam & Podolsky 1969, Julian 1971, Blinks et al. 1978, Lopez et al. 1981, Taylor et al. 1982) may compensate to some extent for the greater sensitivity to active shortening during this phase. It is clear, however, that the free calcium concentration is not high enough to entirely eliminate the shortening induced depression.

E. The depressant effect of active shortening in the anterior byssus retractor muscle (ABRM) of the mollusc *Mytilus edulis* (paper IV)

The depressant effect of active shortening during phasic contraction in ABRM appeared to be considerably smaller than that obtained in vertebrate striated muscle. A given amount of shortening performed during the rising phase of an optimal phasic contraction in ABRM was found to result in a force depression that amounted to less than one tenth of that obtained during the rising phase of a twitch in vertebrate striated muscle (paper III).

However, the shortening effect in ABRM and in vertebrate skeletal muscle exhibited qualitative similarities. Thus, the shortening induced depression obtained in ABRM was entirely reversible (lifetime 8-9 s; Fig. 3) and its magnitude was related to the degree of activation of the muscle (Table 2). These findings are in accordance with observations made in vertebrate skeletal muscle (Edman 1975, 1980).

The reversibility of the depressant effect would seem to exclude a non-uniform distribution in length of the contractile units as a mechanism underlying the shortening effect. The fact that the shortening induced depression: 1. is long-lasting, 2. does not disappear merely by restimulation of the muscle and 3. does not affect the total duration of the contractile response would seem to exclude that the shortening effect is due to altered kinetics of the release and re-uptake of activator-calcium in the excitation-contraction coupling. As in vertebrate striated muscle there is reason to believe that active shortening affects the myofilament system

per se (see further above).

It is considered that ABRM is essentially regulated by the myosin and its associated light chains (Kendrick-Jones et al. 1970, Szent-Györgyi et al. 1973, Lehman & Szent-Györgyi 1975, for review see Kendrick-Jones & Scholey 1981). However, the presence of troponin-like proteins has recently been indicated in several mollusc muscles (Goldberg & Lehman 1978, Lehman 1981). Therefore, the possibility exists that calcium regulates contraction of ABRM both via the myosin and, as in vertebrate striated muscle, through the actin-linked troponin-tropomyosin complex.

The depressant effect of active shortening in ABRM may be related to alteration of the troponin-tropomyosin complex. This is in line with the qualitative similarities and the quantitative difference of the shortening effect between the mollusc muscle and vertebrate striated muscle. However, the possibility exists that the shortening induced depression is due to an effect on the myosin-linked regulatory complex. If so, the myosin-linked system in ABRM would be less sensitive to active shortening than the troponin-tropomyosin system in vertebrate skeletal muscle.

SUMMARY AND CONCLUSIONS

Active shortening is known to exert a depressant effect on the contractile behaviour in vertebrate striated muscle. The purpose of the present study has been to further elucidate the mechanism underlying the shortening effect.

The magnitude of the depressant effect of a given amount of shortening was critically dependent on the amount of calcium around the myofilaments when shortening occurred. Addition of the twitch potentiating drugs diazepam, pentobarbital or 4-aminopyridine caused a marked reduction or a complete elimination of the shortening effect obtained during twitch contraction in the isolated intact muscle fibre. Conversely, in the presence of dantrolene, an agent that is known to reduce the isometric tension, the shortening induced depression was augmented. Similarly, the depressant effect of active shortening in the chemically skinned fibre preparation was inversely related to the free calcium concentration of the surrounding medium. These findings support the view that active shortening leads to reduced affinity of the regulatory troponin-tropomyosin complex for activator-calcium.

An increase in tonicity of the extracellular medium reduced the depressant effect of shortening, while a decrease in tonicity led to augmentation of the shortening effect. The tonicity induced alterations of the shortening effect were not readily explainable by changes in fibre width. Therefore, it seemed most likely that alteration in intracellular ionic strength influenced the depressant effect of active shortening. This conclusion was corroborated by observations made in membrane free preparations of vertebrate skeletal muscle fibres. In these preparations it was demonstrated that the magnitude of the shortening effect was inversely related to the ionic strength of the medium surrounding the myofibrils. These findings were interpreted in terms of feedback influence of the myosin cross-bridges upon the binding of calcium to troponin. Ionic strength is known to affect the speed of dissociation of heavy meromyosin from actin. Changes of the intracellular ionic strength may therefore be expected to modulate the feedback influence of the cross-bridges and, hence, the degree of activation of the contractile system.

The magnitude of the shortening effect produced in the skinned fibre preparation was directly related to the magnesium ion concentration of the surrounding medium. It is suggested that this effect is related to the influence of magnesium on the conformational state of troponin. Increased binding of magnesium may render the regulatory troponin-tropomyosin system more sensitive to the effect of active shortening.

The isometric tension was found to be slightly higher during a tetanus after addition of dantrolene than during a twitch that was potentiated by pentobarbital. However, the depressant effect of a given amount of shortening was considerably smaller in the latter case. Furthermore, the twitch-potentiating drug 4-aminopyridine had the ability to completely eliminate the shortening induced depression during a twitch while the shortening effect during tetanic contraction was reduced but not entirely neutralized. These observations would seem to suggest that the contractile system is more sensitive to active shortening after attainment of steady state force production than during development of contractile activity.

In the anterior byssus retractor muscle (ABRM) of the mollusc Mytilus edulis the depressant effect of active shortening was smaller than in vertebrate striated muscle. However, the shortening effect exhibited qualitative similarities with the effect obtained in skeletal muscle. An effect upon the calcium-regulatory complex of the muscle was regarded as the most plausible mechanism underlying the shortening effect. ABRM is considered to be essentially regulated by the myosin and its associated light chains. The less pronounced shortening effect observed in this mollusc muscle, as compared to that obtained in vertebrate striated muscle, is suggested to be related to the different calcium-regulatory systems in these two muscles.

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Shortening induced deactivation of skinned fibres of frog and mouse striated muscle

M. C. EKELUND and K. A. P. EDMAN

Department of Pharmacology, University of Lund, S-223 62 Lund, Sweden

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The depressant effect of active shortening, previously established in intact muscle fibres, was studied during calcium induced contractures of chemically skinned fibres from the semitendinosus muscle of *Rana temporaria* and the psoas muscle of the mouse. The decrease in contractile activity was determined by comparing the rate of force redevelopment (at a given tension level) after a large (test) and a small (control) release step. Under standard experimental conditions (ionic strength: frog 135 mM, mouse 190 mM; Ca^{2+} 3.0 μM ; Mg^{2+} : frog 25 μM , mouse 100 μM ; MgATP^{2-} : frog 1.0 mM, mouse 2.0 mM) active shortening of 0.15 μm per sarcomere (in excess of control release) reduced the contractile activity by approximately 50% of the control in both frog and mouse muscle fibres. Full contractile activity was regained within <4 s during isometric activity after the shortening phase. The depressant effect of shortening was steadily reduced, to almost complete disappearance of the effect, by increasing the free calcium concentration within the range 1.5–12.0 μM . Similarly, an increase in ionic strength from 105 to 235 mM reduced the depressant effect by approximately 40%. In contrast, there was a progressive enhancement of the shortening effect as the magnesium ion concentration was increased from 25 to 590 μM . It is proposed that interaction between the myosin cross-bridges and the thin filament during sarcomere shortening leads to a decrease in troponin-calcium binding resulting in a temporary deactivation of the contractile system.

Key words: Skeletal muscle, skinned muscle fibres, shortening induced deactivation, calcium, magnesium, ionic strength

Shortening during activity of vertebrate skeletal muscle causes a transitory decrease of the muscle's ability to produce force. This depressant effect of shortening has been demonstrated in whole muscle *in situ* in the body (Joyce & Rack 1969, Joyce, Rack & Westbury 1969) as well as in isolated whole muscle (Jewell & Wilkie 1960, Briden & Alpert 1972) and single muscle fibres (Edman & Kiessling 1971, Edman 1975, 1980).

The decrease in active force has been shown to be quantitatively related to the amount of preceding shortening and to be independent, over a wide range, of the speed of shortening and of the load and work output during the shortening phase (Edman 1975). The depressant effect is longlasting, its lifetime being of the order of one second, but it is noteworthy that the effect does eventually disappear completely during continued stimulation of the fibre (Edman 1975). The evidence obtained so far

suggests that the depressant effect of shortening is an entirely physiological phenomenon which reflects the normal behaviour of the contractile system in response to filament sliding. Further insight into the nature of the shortening effect may therefore contribute relevant information to the elucidation of the molecular events involved in the sliding filament process.

In the present paper we describe experiments aimed at exploring the shortening effect in more detail in chemically skinned fibres of frog and mouse skeletal muscle. The evidence obtained supports the view that active shortening leads to a transitory deactivation of the contractile system, an effect which can be enhanced by increasing the magnesium ion concentration and inhibited by raising the concentration of free calcium in the myofibrillar space. Results will also be presented to show that deactivation by active shortening is critically

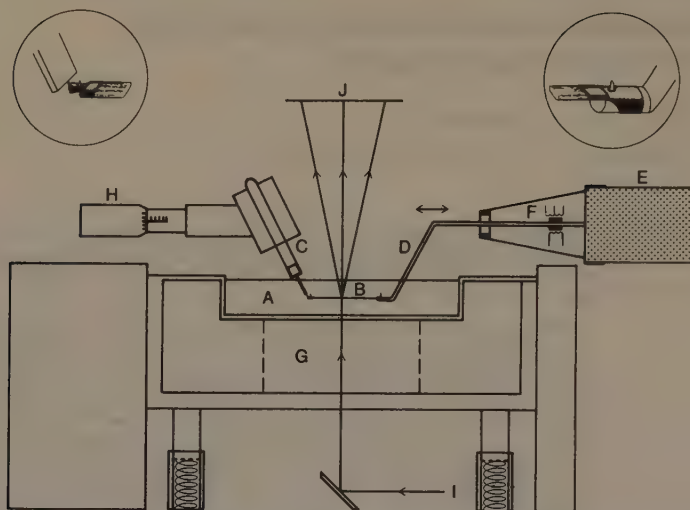


Fig. 1. Schematic illustration of experimental arrangement. (A) Side view of muscle chamber. (B) muscle fibre; (C) tension transducer; (D) shaft movable in the horizontal plane; (E) electromagnetic puller; (F) displacement transducer; (G) air space for passage of laser beam; (H) micrometer screw; (I) direction of laser beam; (J) screen for recording laser diffraction pattern. Insets: Detail of fibre attachment to force transducer (left) and puller arm (right). Note that the fibre ends are clamped by an aluminium foil and that the side parts of the foil are folded around the end of the puller shaft and the steel hook of the transducer. Diagram not drawn to scale.

dependent on the ionic strength of the intracellular medium.

METHODS

Preparation and mounting. The experiments were performed on isolated segments of single fibres prepared from the semitendinosus muscle of *Rana temporaria* and the psoas muscle of the mouse. The muscle, after being removed from the animal, was kept at room temperature in a 'high potassium solution' (Julian 1971) containing 100 mM KCl, 2 mM EGTA and 10 mM NaH_2PO_4 - Na_2HPO_4 buffer (pH 7.0). A bundle of the muscle was cut free and subdivided into finer bundles by careful teasing until, eventually, single fibre segments (approximately 5 mm long) could be isolated.

The isolated fibre segments which remained relaxed in the dissection solution were transferred into an 'extraction solution' containing 30% (v/v) glycerol, 2 mM EGTA and 10 mM NaH_2PO_4 - Na_2HPO_4 buffer (pH 7.0).

The fibres were stored in this solution at +4°C for approximately 1 h. In a few experiments the fibres were kept in the 'extraction solution' overnight with no apparent difference in results. For transportation between different solutions the fibres were carried on a thin glass rod containing a surface layer of solution.

After glycerol extraction the fibres were briefly rinsed in the 'high potassium solution' and were thereafter stored in the 'relaxing solution' (composition, see below) at +4°C. The glycerol extracted fibres were divided into segments of approximately 2 to 3 mm length and clips of aluminium foil were attached to either end of the segment so that a 1-2 mm long portion of the fibre was left free between the clips (Fig. 1).

The fibre provided with the clips was transported in a small Perspex vessel containing 'relaxing solution' to the experimental chamber. The preparation was mounted in the relaxing solution between a force transducer and a shaft extending from an electromagnetic puller (Fig. 1).

The aluminium clips were provided with a hole to fit a small hook of steel wire (0.10 mm thickness) on the force transducer and the puller arm, respectively. The aluminium foils were folded around the hooks so that the clips became firmly attached to their mounting sites to ensure that the fibre did not alter its position when the bath was exchanged (see below).

After mounting, the fibre was immersed for 10 to 15 min in a 'relaxing solution' containing 0.5% (w/v) Brij 58. It was thereafter kept in plain 'relaxing solution'.

Muscle chamber. Four identical chambers, each containing 4 ml solution, were milled into a Perspex block (Fig. 1) that could be lowered and translated so that any of

Table 1. Stability constants

1. $KATP^{3-}$	=8	7. $MgHATP^{1-}$	= 3.8×10^2	12. H_3EGTA^{1-}	= 4.8×10^2
2. $CaATP^{2-}$	= 3.0×10^4	8. $MgEGTA^{2-}$	= 1.6×10^5	13. H_2EGTA	= 1.0×10^2
3. $CaHATP^{1-}$	= 3.0×10^2	9. $MgHEGTA^{1-}$	= 2.3×10^3	14. $HATP^{3-}$	= 9.4×10^6
4. $CaEGTA^{4-}$	= 3.2×10^{11}	10. $HEGTA^{3-}$	= 8.7×10^9	15. H_2ATP^{2-}	= 1.7×10^4
5. $CaHEGTA^{1-}$	= 2.1×10^5	11. H_2EGTA^{2-}	= 1.6×10^9	16. $Himid^{1+}$	= 2.9×10^7
6. $MgATP^{2-}$	= 4.0×10^4				

ATP = Adenosine triphosphate, EGTA = Ethylene-glycol-bis-(β aminoethyl ether)-N,N'-tetraacetic Acid, Imid = Imidazole.

the chambers could be used for immersion of the fibre. The side-walls of the chambers were surrounded by a jacket for circulation of a thermostatically controlled water-glycol solution from a Colora Ultrathermostat. The floor of the chambers was made of a glass slide for passage of a laser beam.

Temperature. The temperature was maintained constant to $\pm 0.2^\circ\text{C}$ within any given experiment and varied between 1.6 and 2.2°C in the different experiments on frog muscle fibres and between 14.5 and 15.5°C in the mouse fibre experiments.

Tension recording. A semiconductor strain-gauge transducer (AE 801, Aksjeselskapet Mikroelektronikk) was used as described previously (Edman 1980).

Electromagnetic puller. Controlled length steps were produced by an electromagnetic puller and servo system of the type described previously (Edman 1975). The puller arm (Fig. 1) moved horizontally along the longitudinal axis of the fibre. Its movement was monitored by means of a capacitance or a differential transformer type transducer on the two pullers used during this investigation.

For the purpose of the present experiments relatively slow length changes were used, the rise time being approximately 5 ms. In the standard type of experiment two length steps of different amplitude were applied at an interval of 4 s during contracture of the fibre.

Determination of fibre length and sarcomere spacing. The overall length of the fibre segment was measured at rest as the distance between the edge of the aluminium clips that were attached to either end of the segment. The measurement was made to the nearest 0.025 mm using a Zeiss Stereo III microscope at $40\times$ magnification. Before this measurement the sarcomere length (see below) had been adjusted to $2.25\ \mu\text{m}$.

The sarcomere spacing at rest was determined from the laser diffraction pattern (Cleworth & Edman 1972) that was displayed on a calibrated screen placed horizontally above the muscle chamber. A distinct pattern was regularly observed at rest and a clear first-order line could be seen throughout the contraction although it became wider and less distinct as contraction proceeded. Changes in sarcomere length during activity were calculated from the changes in overall fibre length and the sarcomere spacing at the onset of puller movement.

Recording and measurement of responses. The signals from the tension and displacement transducers were displayed on a Tektronix 5103 N storage oscilloscope and photographed on 35 mm orthochromatic film. Measurements from the film records were made in a Nikon model

6C profile projector at $10\times$ magnification using the stage micrometer readings. The rate of force redevelopment after shortening was measured to the nearest minute of arc on the Nikon vernier scale after aligning one of the crosshairs of the projector with the myogram so as to obtain the tangent of the myogram at a selected point (see further under Results).

Solutions. The solutions used for relaxation and contraction had the following composition (values refer to total concentration, mmol/l): 'Relaxing solution' (Julian 1971): KCl 100, $MgCl_2$ 1, ATP 4, EGTA 2, Imidazole 10 (pH 7.0).

'Contracture solution': The standard contracture solution had the following composition: KCl 100, $MgCl_2$ 1, ATP 4, EGTA 4, Imidazole 10, $CaCl_2$ 3.7 (pH 7.0).

Table 2. Concentration of Ca^{2+} , Mg^{2+} $MgATP^{2-}$ and ionic strength in the contracture solutions used in sections A–D under Results

Section under results	Contracture solution			Ionic strength (mM)
	Ca ²⁺ (μM)	Mg ²⁺ (μM)	MgATP ²⁻ (mM)	
<i>Frog</i>				
A	1.5 3.0	25	1.0	135
B	1.5 3.0 6.0 12.0	25	1.0	135
C	3.0	25 118 590	2.5	130
D	3.0	25	1.0	105 135 235
<i>Mouse</i>				
A	3.0	100	2.0	190
B	1.5 3.0 6.0 12.0	100	2.0	190

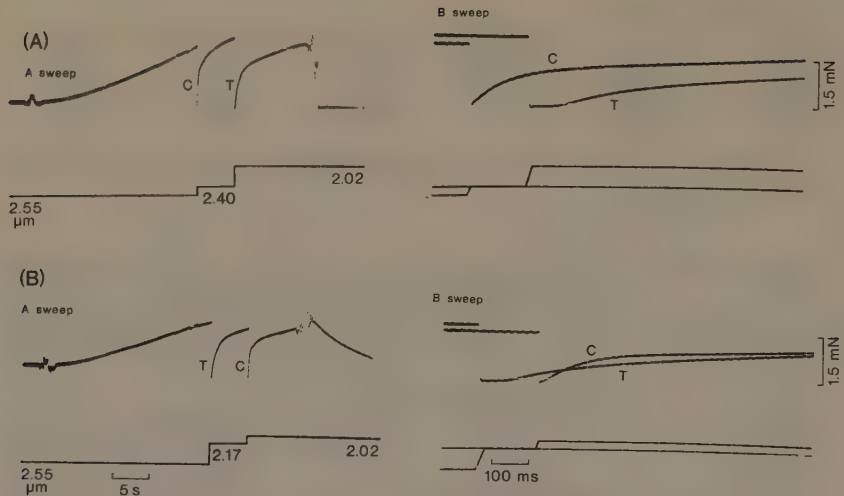


Fig. 2. Redefinition of force after small (control) and large (test) release steps (traces C and T, respectively) during calcium induced contracture of chemically skinned fibre of the frog. A-sweep (left panel) shows the entire course of contracture on slow time base (5 s/div). B-sweep (right panel) shows release steps and force redevelopment

on fast time base (100 ms/div). The order of the small and large release steps is reversed in (A) and (B). Note that the rate of force redevelopment is lower after the large shortening (T), at any given tension level, within one to two seconds after the onset of force redevelopment.

The total concentrations of KCl, $MgCl_2$, $CaCl_2$ and ATP of the contracture solution were varied in different series of experiments to give the concentrations of Ca^{2+} , Mg^{2+} , $MgATP^{2-}$ and ionic strength specified in Table 2. For calculation of the concentrations of free ions and complexes in the solution a computer program was utilized using the stability constants given in Table 1 (for references, see Godt 1974).

The constants for complexes Nos 2, 4, 6, 7, 10, 11, 14, 15 and 16 in Table 1 refer to 4°C. For complexes Nos 1, 3, 5, 8, 9, 12 and 13 the stability constants refer to room temperature (20–25°C) and no temperature correction has been made in these cases due to lack of information about activation energy for use in the Arrhenius equation. The stability constants listed in Table 1 have been used in experiments on both frog (2°C) and mouse (15°C) muscle fibres.

The use of absolute stability constants for determination of individual ions and ion complexes in a composite solution involves some uncertainty. The error introduced in this way is likely to outway the actual differences in binding stability that exist within the range of temperature used in these experiments. However, this uncertainty concerning the true ionic concentrations is not critical for the present investigation, as the main purpose has been to evaluate effects produced by changes in ionic concentrations.

Experimental procedure. After mounting and treatment in the Brij 58 solution (see above) the fibre was immersed in the 'relaxing solution'. The sarcomere length was adjusted to 2.25 μm , and the overall length of the fibre segment measured. The fibre was thereafter stretched to a sarcomere length of 2.45 μm (or 2.55 μm in a few experiments under section A, Results) and the amplitude of the release steps to be used during the subsequent contractions was tested out.

Contracture was induced by immersing the fibre in the 'contracture solution'. At a point near maximum isometric tension the puller was activated to produce two steps of release (0.15 and 0.30–0.40 μm /sarcomere shortening, respectively) at an interval of four seconds.

The 4-s delay of the second release step enabled the fibre to redevelop tension to approximately the same level as existed before the first step. Generally it was possible to repeat several contractions in a given fibre without any noticeable deterioration of the response. In the majority of the mouse psoas fibre experiments (14.5–15.5°C) the interval between the two release steps was 2.5 s. This interval enabled full recovery of tension under these conditions.

Fibres were excluded: 1) if they exhibited a resting tension that was larger than 5% of the maximal active tension at 2.45 μm sarcomere length, 2) if the diffraction pattern was diffuse at rest and/or disappeared during the

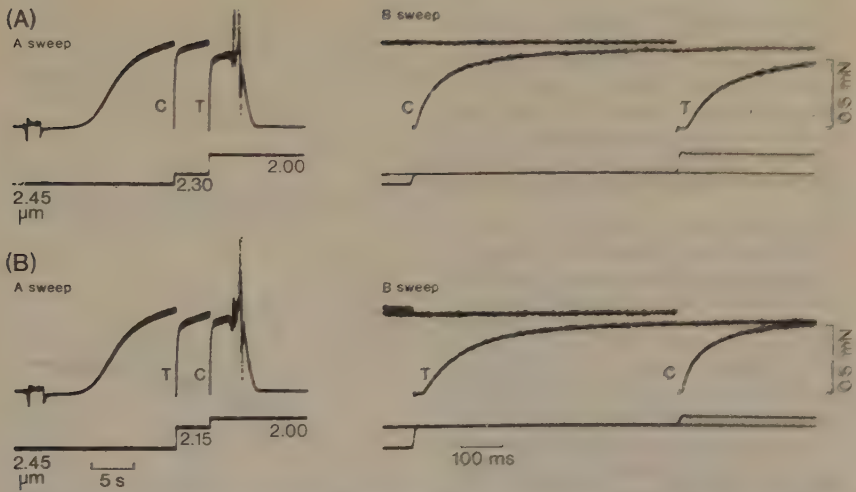


Fig. 3. Redevelopment of force after small (control) and large (test) release step (traces C and T respectively) during calcium induced contracture of chemically skinned fibre of the mouse. For further information, see legend of Fig. 2.

rising phase of the contracture, 3) if the force redevelopment after the release was incomplete and 4) if any visible nick or inhomogeneity appeared along the fibre segment.

Student's *t*-test was used for determination of statistical significance.

RESULTS

A. Force depression after active shortening

The influence of preceding active shortening on the fibre's ability to develop force was studied during calcium induced contractures of chemically skinned muscle fibres of the frog using the procedure illustrated in Fig. 2.

The fibre was first allowed to develop force at a sarcomere length of 2.4–2.5 μm until nearly maximum tension was attained (Fig. 2A). At this point the fibre was quickly released by 0.15 μm /sarcomere. This amplitude of release caused a drop in tension to zero and an immediate redevelopment of force. After a fixed time of 4 s, when full contractile force had been restored, a new, larger release (0.30–0.40 μm /sarcomere) of the same speed was applied. The magnitude of the second release step was more than sufficient to reduce tension to zero, which means that the fibre was allowed to shorten

by an additional amount at zero load to take up the slack. Fig. 2B shows the same procedure repeated in the same fibre but using a reversed order of the small and large release steps. It is clearly seen from the fast sweep records of Fig. 2A, B (right panels) that the rate of force redevelopment was smaller after the large (test) release than after the small (control) release at any given tension level within 1–2 s after the shortening phase. However, the same final tension was attained after both amplitudes of release. These findings suggest that, similar to the situation in the intact fibre (Edman 1975, 1980), active shortening depresses the fibre's ability to produce force and that this depressant effect eventually disappears during continued isometric activity.

The magnitude of the depressant effect was determined by measuring the rate of force redevelopment (V_T and V_C) when the fibre passed a given force level after the test and control step, respectively. As a standard procedure in these experiments the rate of force redevelopment was measured at 25% of maximum contracture tension (see Fig. 5 inset). This point of measurement occurred on the steep portion of the force myogram. The fact that the measurements refer to the same tension

level in test and control implies that the load on the contractile system was the same in both cases. A difference in rate of force redevelopment measured in this way therefore reflects a difference in the fibre's contractile activity at these points. The depressant effect was determined as the ratio $(V_C - V_T)/V_C$ and was related to the difference in sarcomere shortening during the test and control steps.

The depressant effect of shortening was recorded in a number of frog muscle fibres in which the release sequence was arranged as control-test and test-control in the same preparation. The free calcium concentration varied between 1.5 and 6.0 μM in these experiments. V_T and V_C were measured at 25% of maximum contracture force as described above. The depressant effect of 0.15–0.22 μm /sarcomere shortening was found to be $61 \pm 13\%$ (SD, $n=28$) and there was no significant difference between results obtained with the two release protocols (paired observations, $p>0.9$). This lack of dependence on the relative order of the test and control releases clearly shows that the depressant effect was fully reversed within the time interval (4 s) that separated the first and the second release steps.

It was of interest to find out if a depressant effect of shortening also occurred in skinned mammalian muscle fibres. To this end a series of experiments similar to those described in Fig. 2 was performed on chemically skinned psoas muscle fibres of the mouse. Typical results are illustrated in Fig. 3 A, B. It is evident that active shortening reduced the force producing capability of the mouse muscle fibre in a manner very similar to that observed in the frog muscle preparation. Compared with the frog muscle fibres the mouse psoas preparations exhibited a more stable mechanical behaviour during repeated contractures. These fibres could generally perform a greater number of contraction-relaxation cycles before any of the above exclusion criteria (see Methods) appeared.

B. The influence of Ca^{2+} concentration on depressant effect of shortening

Evidence obtained in studies of intact fibres (Edman 1980) suggests that the magnitude of force depression after active shortening is related to the state of activation of the contractile system when shortening occurs. It was therefore of interest to find out if the depressant effect of shortening ob-

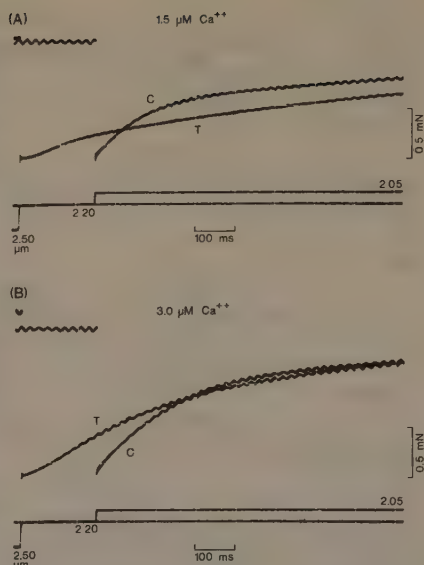


Fig. 4. Superimposed oscilloscope records illustrating redevelopment of force after small (control, C) and large (test, T) release step in skinned muscle fibre at 1.5 (A) and 3.0 (B) μM Ca^{2+} . Note that the decrease in rate of force redevelopment after the large test release step is less pronounced at the higher calcium concentration.

tained in the skinned preparation was dependent on $[\text{Ca}^{2+}]$. In these and subsequent experiments the control and test release steps were standardized to 0.15 and 0.30 μm per sarcomere, respectively, from an initial sarcomere length of 2.45 μm .

Fig. 4 illustrates recordings for determination of the depressant effect of shortening at two different concentrations of free calcium, 1.5 and 3.0 μM . The decrease in contractile activity is indicated by the difference in rate of force redevelopment after test and control release, respectively. As can be seen the depressant effect of shortening was lower at the higher calcium concentration. Results from similar experiments in which $[\text{Ca}^{2+}]$ was varied between 1.5 and 12.0 μM are summarized in Fig. 5. The magnitude of the depressant effect varied a great deal from fibre to fibre at any given calcium concentration. However, for each individual fibre there was a clear reduction of the depressant effect as the calcium ion concentration was increased between

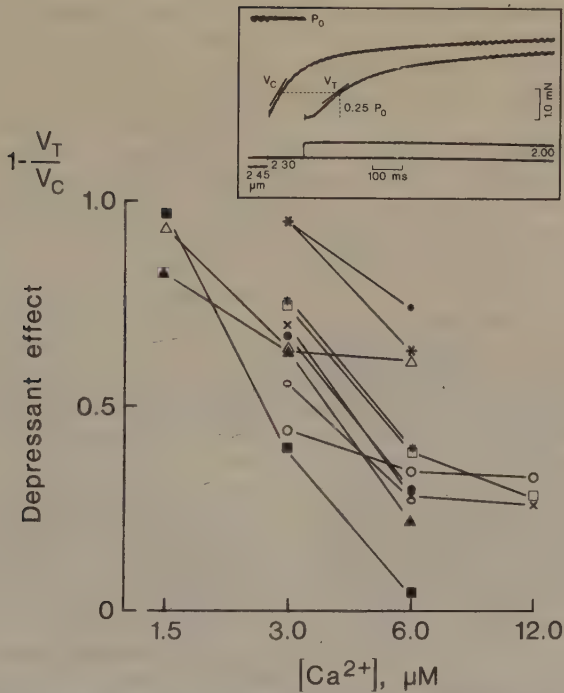


Fig. 5. Depressant effect of active shortening of skinned muscle fibres of the frog related to free calcium concentration. The depressant effect was measured as decrease in rate of force redevelopment at 25% of maximum isometric force as illustrated by inset. Two series of experiments covering different ranges of calcium concentration are shown. Each set of data denoted by the same symbol is from a single preparation.

1.5 and 6.0 μM . The effect recorded at 6.0 μM calcium was 31 (range: 3–66) % of the effect measured at 1.5 μM calcium (3 fibres) and 57 (range: 39–78) % of the effect measured at 3.0 μM calcium (8 fibres).

Attempts were made to investigate the shortening effect at calcium ion concentrations higher than 12 μM . However, under these conditions the fibres generally exhibited signs of damage during the contraction resulting in incomplete redevelopment of force after the first or second release steps.

The influence of calcium concentration on the depressant effect of shortening was also investigated in the skinned psoas muscle preparation of the mouse. The results of such measurements are pre-

sented in Fig. 6. In similarity with the results obtained in the frog fibre experiments an increase in free calcium concentration from 1.5 to 12.0 μM caused a marked decrease of the shortening effect. At 6.0 μM $[\text{Ca}^{2+}]$ (5 fibres) the depressant effect of shortening was 7–41 % of the effect recorded at 1.5 μM $[\text{Ca}^{2+}]$. There was only a slight further reduction of the shortening effect by increasing the calcium ion concentration from 6.0 to 12.0 μM .

C. The influence of free Mg^{2+} concentration on depressant effect of shortening

These experiments were performed in order to investigate if variation in magnesium ion concentration influenced the depressant effect of shortening.

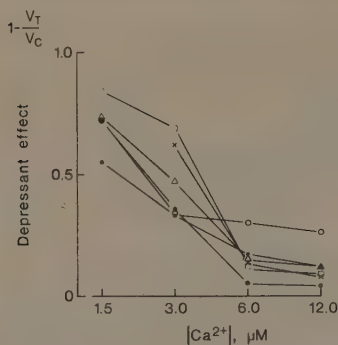


Fig. 6. Depressant effect of active shortening of skinned muscle fibres of the mouse related to free calcium concentration. Measurement of depressant effect performed as in Fig. 5 (inset). Each set of data denoted by the same symbol is from a single preparation.

The same experimental protocol was used as described in section B. The magnesium ion concentration was varied between 25 and 590 μM keeping the concentrations of MgATP^{2-} (2.5 mM) and free calcium (3.0 μM) and the ionic strength (130 mM) constant.

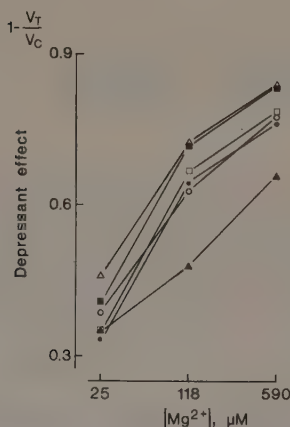


Fig. 7. Depressant effect of active shortening at varied magnesium ion concentration. Measurement of depressant effect performed as in Fig. 5 (inset). Data points denoted by a given symbol are from the same preparation.

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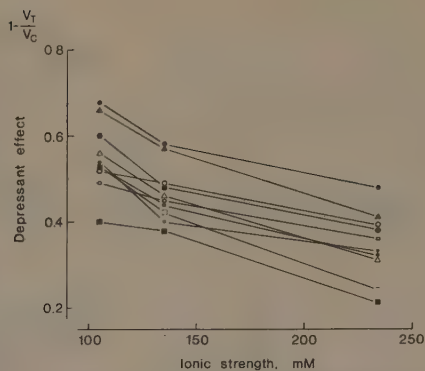


Fig. 8. Depressant effect of active shortening at varied ionic strength. Measurement of depressant effect performed as in Fig. 5 (inset). Data denoted by the same symbol refer to a single preparation.

Fig. 7 summarizes results obtained in six experiments. Raising the magnesium ion concentration caused a continuous increase of the depressant effect of shortening. The movement effect can be seen to approximately double as the $[\text{Mg}^{2+}]$ was increased from 25 to 590 μM .

D. The influence of ionic strength on depressant effect of shortening

Fig. 8 shows how the depressant effect of shortening varies with a change in ionic strength. Here $[\text{Ca}^{2+}]$, $[\text{Mg}^{2+}]$ and $[\text{MgATP}^{2-}]$ were kept constant at 3.0 μM , 25 μM and 1.0 mM, respectively, while the ionic strength was varied from 105 to 235 mM. The degree of force depression by shortening was inversely related to the ionic strength. An increase in ionic strength from 105 to 235 mM reduced the movement effect by approximately 40%.

DISCUSSION

Comparison between results obtained in intact fibres and skinned preparations

Active shortening of vertebrate striated muscle causes a transitory reduction of the muscle's ability to produce force. This phenomenon has previously been explored in isolated intact skeletal muscle fibres of the frog (Edman 1975, 1980, 1981), and results from such studies suggest that the depressant effect of shortening is based on a change in the

contractile system itself leading to a temporary reduction of the mechanical response. The present results support this view by demonstrating that a depressant effect of shortening also occurs in skinned single fibre preparations from both frog and mouse skeletal muscle. In these preparations the free calcium concentration can be carefully controlled. With the present experiments it is thus possible to exclude that the decrease in mechanical performance after shortening is due to altered kinetics of the release and reuptake of activator-calcium in the excitation-contraction coupling. The magnitude of force depression by shortening in the skinned preparation is comparable to that recorded in intact fibres (Edman 1975). Thus, at a submaximum calcium ion concentration, $0.15 \mu\text{M}$ /sarcomere shortening was found to reduce the contractile activity to approximately 50% of the control value. However, the magnitude of the depressant effect was found to be dependent on both the ionic composition and the total ionic strength of the surrounding medium (see further below).

The decrease in contractile activity after shortening persists over a relatively long time, in the order of one second, in both intact and skinned fibres. It is of interest to note, however, that the contractile potential is fully restored during continued isometric recording. This feature of the shortening effect would seem to rule out that the reduced mechanical output after shortening is due to non-uniform distribution of sarcomere length. A disorder of the sarcomere length at the end of shortening would not disappear but would probably become more severe during the subsequent isometric phase. Thus, if the decrease in mechanical performance after shortening were due to sarcomere non-uniformity the effect of shortening would persist, or even increase, during the remainder of the activity period. The transient nature of the depressant effect, with attainment of the same final tension, may therefore be taken as evidence that the transitory decrease in contractile activity that occurs after shortening is a property shown by all sarcomeres along the fibre.

As demonstrated in experiments on intact fibres (Edman 1980) the shortening effect does not include any change in V_0 , the velocity of shortening at zero load. This finding excludes the possibility that force depression after shortening is due to increased passive resistance to filament sliding. The shortening effect thus seems to reflect a true depression of the force producing capability of the contractile sys-

tem. The constancy of V_0 furthermore suggests that the shortening effect is not attributable to altered cross-bridge kinetics or to recruitment of a particular class of cross-bridges that would have a slow turnover rate (Gulati & Podolsky 1981).

Nature of the depressant effect of shortening

Evidence obtained in previous studies of intact muscle fibres suggested that the degree of force depression after active shortening is inversely related to the level of activation of the contractile system during the shortening phase (Edman 1980, 1981). The present results substantiate this conclusion by demonstrating that the shortening effect is gradually reduced as the calcium ion concentration is raised in the skinned fibre. By increasing the free calcium concentration to $6.0 \mu\text{M}$ it was possible to reduce the depressant effect to 3–66% (frog) and 7–41% (mouse) of the value recorded at $1.5 \mu\text{M}$ calcium. The finding that the shortening effect is diminished by increasing the calcium ion concentration is evidence in favour of the idea (Edman 1980) that active shortening temporarily deactivates the contractile system by reducing the affinity of the binding sites for calcium on the thin filament. Such a decrease in calcium affinity would affect the mechanical response only as long as the contractile system is below the level of mechanical saturation. According to this view the effect of shortening would become smaller and smaller with increasing concentrations of activator-calcium and would be expected to disappear as the calcium ion concentration is raised well above the mechanical saturation level. For reasons given earlier (see Results) it was not practicable to test calcium concentrations that were clearly supramaximal. It is evident, however, that the calcium concentrations used were sufficiently high to almost completely eliminate the shortening effect in some fibres.

The idea that the affinity of troponin for calcium is modulated during contraction is supported by biochemical observations. Thus, it has been demonstrated *in vitro* that calcium binding to troponin in isolated thin filaments is markedly increased as myosin heads are attached to the actin moieties (Weber & Murray 1973). This local feedback control of calcium binding exerted by the myosin cross-bridges has been interpreted to mean that there exists a cooperative activity between the proteins of the calcium regulated thin filament (Weber & Murray 1973, Adelstein & Eisenberg 1980). The

depressant effect of shortening may be another manifestation of such a cooperative activity. Thus, interaction between the myosin bridges and the actin filament *during sliding* may lead to a temporary decrease in calcium affinity and, therefore, to a transitory deactivation of the contractile system. The proposed mechanism is in line with the observation (Ekelund & Edman, unpublished) that the contractile activity is only slightly reduced by shortening in the anterior byssus retractor muscle of *Mytilus edulis* which is mainly myosin regulated (Kendrick-Jones et al. 1970, Lehman & Szent-Györgyi 1975, Lehman 1981).

Magnesium and ionic strength are, in addition to calcium, important factors in the development of the shortening effect. An increase in magnesium ion concentration from 25 to 590 μM was found to approximately double the depressant effect of shortening (Fig. 7). Magnesium as well as calcium binds both to troponin and to myosin light chains and there is evidence that the two ions compete for certain binding sites in these proteins (Bagshaw 1977, Bremel & Weber 1975, Holroyde et al. 1979, Potter & Gergely 1975, Potter et al. 1981, Robertson et al. 1981, Weber & Murray 1973). This competition, however, does not concern the 'calcium-specific sites' that are directly involved in calcium-induced activation of the contractile system (Potter & Gergely 1975, Potter et al. 1981, Robertson et al. 1981). It has been proposed that magnesium by binding to the calcium-magnesium-sites modulates the conformational state of troponin (Potter et al. 1981, Levine et al. 1978). This function of magnesium may be of relevance in explaining the enhancement of the shortening effect that was observed as the magnesium ion concentration was raised. Increased binding of magnesium may render troponin more responsive to feedback influence of the myosin cross-bridges. Increased magnesium ion concentration may thus enhance the conformational change of troponin that is proposed to underlie the depressant effect of shortening (see earlier).

Increasing ionic strength was found to progressively inhibit the depressant effect of shortening. This is an interesting finding in view of the fact that increased ionic strength has been shown to specifically increase the speed of dissociation of heavy meromyosin from actin (Eisenberg & Moos 1970, Rizzino et al. 1970). This result supports the idea that the shortening effect is a direct consequence of the actin-myosin interaction during shortening, i.e.

that deactivation is an integral part of the sliding-filament process in actin regulated contractile systems. The kinetic model advanced by Adelstein & Eisenberg (1980) suggests various possible ways by which cross-bridge cycling may affect calcium sensitivity by changing the troponin-calcium binding equilibrium. A possibility worth considering in the light of the present results would be that deactivation by shortening is effectuated by cross-bridges that are attached to the thin filament over a sufficiently long time to end up in a braking position during the sliding process (see further Huxley 1957). The braking force exerted on the thin filament by such cross-bridges, or indeed the peculiar angular configuration of these bridges (cf. Godt & Maughan 1981) may be of relevance for the induction of altered calcium sensitivity in response to filament sliding. An increase in ionic strength would be expected to reduce the number of cross-bridges in 'holding' position and, therefore, to diminish the inhibitory influence on the troponin-calcium binding that may be produced by such cross-bridges during the sliding filament process.

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THE INFLUENCE OF VARIED TONICITY OF THE EXTRACELLULAR MEDIUM ON THE DEPRESSANT EFFECT OF ACTIVE SHORTENING IN VERTEBRATE STRIATED MUSCLE

M.C. Ekelund

Department of Pharmacology, University of Lund, S-223 62 Lund, Sweden

The depressant effect of active shortening was studied during isometric twitch contraction in intact single muscle fibres of the frog at varied tonicity of the extracellular medium. The shortening effect was calculated as the difference in peak redeveloped force after a small (control) and a larger (test) release step and was expressed in per cent of the isometric tetanic force. The solutions were made hypertonic by addition of sucrose (relative tonicity 1.22T and 1.44T) and hypotonic by reduction of NaCl (relative tonicity 0.81T and 0.62T). The shortening induced depression decreased from $13.0 \pm 1.2\%$ in normal Ringer solution to $7.8 \pm 1.3\%$ after immersion of the fibre in 1.22T solution (mean $\pm$ S.E., $n = 7$). This reduction of the depressant effect by shortening was slightly less than half the size of that obtained in 1.44T solution. An increased force depression by shortening, from $12.7 \pm 1.2\%$ to $17.1 \pm 1.5\%$ (mean $\pm$ S.E., $n = 7$), was obtained when normal Ringer was replaced by 0.81T solution. This enhancement was further augmented in 0.62T solution. Experimental evidence is presented supporting the view that the influence of tonicity on the depressant effect of shortening is not due to tonicity induced changes in fibre width. The effect of varied tonicity on the shortening induced depression appears to be essentially related to alterations in intracellular ionic strength.

Altered tonicity of the extracellular medium has been shown to affect the contractile behaviour of muscle. In a hypertonic solution, both active tension and maximum velocity of shortening are decreased. Conversely, at reduced tonicity of the extracellular medium isometric force production and shortening velocity are both increased (e.g. April et al. 1968, Edman & Andersson 1968, Edman & Hwang 1977). The precise mechanism underlying these contractile changes is still debated. It is likely, however, that the tonicity induced alterations in myofilament lattice width and intracellular ionic strength (Dydyńska & Wilkie 1963, Blinks 1965, Rome 1968, Edman & Hwang 1977, Millman et al. 1981, Gulati & Babu 1982) may both affect the mechanical performance of the contractile system.

Several previous studies have demonstrated that shortening during activity of vertebrate striated muscle has a depressant effect on the muscle's capacity to produce force (Jewell & Wilkie 1960, Joyce & Rack 1969, Joyce et al. 1969, Edman & Kiessling 1971, Briden & Alpert 1972, Dickinson & Woledge 1973). This force depression is transitory and disappears completely during continued isometric activity (Edman 1975). Furthermore, evidence has been presented indicating that the shortening induced depression does not involve altered kinetics of the release and reuptake of activator calcium in the excitation-contraction coupling, sarcomere non-uniformity or increased passive resistance to sliding of the thick and thin filaments. Instead, it is conceivable that the shortening effect is related to a change in the myofilament system itself (Edman 1975, 1980, Ekelund & Edman 1982).

In the present study, experiments have been performed to investigate if the depressant effect of active shortening during twitch-contraction in intact single muscle fibres of the frog is influenced by variations in tonicity of the extracellular medium. For this purpose sucrose was added to normal Ringer solution (1.00T) resulting in a relative tonicity of 1.22T and 1.44T. The hypotonic solutions were obtained by reducing the NaCl concentration from 115.5 mM to 92.4 and 69.3 mM. This resulted in a relative tonicity of 0.81T and 0.62T, respectively. The present investigation shows that altered tonicity of the extracellular medium modulates the shortening effect. Attempts were made to find out if the observed effects were attributable to a change in myofilament lattice width or to altered intracellular ionic strength.

METHODS

Preparation and mounting. Single muscle fibres from the semitendinosus and tibialis anterior muscles of Rana temporaria were used. The mechanical behaviour of fibres obtained from the two different muscles was essentially the same. The technique used for dissection was similar to that described previously (Edman & Kiessling 1971). Clips of aluminium foil were attached to the tendons, close to the fibre-tendon junction. The fibre was thereafter transported in a Perspex vessel containing Ringer solution (composition, see below) to the experimental chamber. The preparation was mounted horizontally in the chamber between a hook of steel wire extending from a force transducer and an identical hook fixed to the tip of a movable arm of an electromagnetic puller. A small hole in the aluminium foil fitted

the hook and the remaining part of the clip was folded around the hook to ensure firm attachment to the mounting site. The rest length of the fibre was set to the desired value by adjusting the position of the puller and/or the force transducer.

Solutions. Five different solutions (I-V) of varied tonicity were used in the present study. The relative tonicity of each solution was estimated by using the formula provided by Dydyńska & Wilkie (1963) for calculation of the osmotic coefficient of sucrose and assuming the osmotic coefficient of NaCl to be 0.93. The estimated values are given within brackets.

I Normal Ringer solution (1.00T) (mM):

NaCl 115.5, KCl 2.0, CaCl_2 1.8, Na phosphate buffer 2.0, pH 7.0.

II Hypotonic Ringer (0.81T):

The same composition as in I except for a reduction of the NaCl concentration to 92.4 mM.

III Hypotonic Ringer (0.62T):

The same composition as solution I with the exception that the NaCl concentration was reduced to 69.3 mM.

IV Hypertonic Ringer (1.22T):

Solution I with addition of 50 mM sucrose.

V Hypertonic Ringer (1.44T):

98 mM sucrose was added to solution I.

Throughout the experiment the solution was perfused through the muscle chamber at a speed of approximately 1.8 ml min^{-1} . The fibre was immersed in one of the above solutions for at least 40 minutes before a series of recordings was carried out. Normotonic Ringer (solution I) was perfused through the muscle chamber between application of the hypo- or hypertonic solutions.

Muscle chamber. A jacket surrounded the muscle chamber (containing 4.5 ml solution) through which a thermostatically controlled water-glycol solution from a Colara Ultrathermostat was circulated. The floor of the chamber was made of a microscope slide allowing passage of a laser beam used for determination of sarcomere spacing (see below).

Temperature. The temperature varied between 2.0 and 3.2°C in the different experiments and was maintained constant to $\pm 0.3^\circ\text{C}$ within any given experiment.

Sarcomere length recording. Laser diffraction technique, essentially the same as previously described (Cleworth & Edman 1972), was used for determination of sarcomere spacing at rest and during activity. A helium-neon continuous-wave laser (Spectra Physics, model 133) was utilized and the diffraction pattern was displayed on a screen placed horizontally above the muscle chamber. By using the formula for light diffraction a scale was calibrated and placed on the horizontal screen. This enabled a direct reading of the sarcomere length from the zero- to first-order line spacing. Changes in fibre length in response to release steps of different amplitude were calculated from the change in overall fibre length and the sarcomere length at the onset of the lever movement.

Determination of fibre length. The distance between the insertions of the fibre to the tendons, i.e. the overall fibre length, was measured to the nearest 0.05 mm in a Zeiss Stereo II microscope at 10 x magnification.

Stimulation. The fibres were stimulated by passing current through two platinum plate electrodes. These were placed symmetrically on either side of the fibre at a distance of approximately 1.5 mm from the preparation. The stimulus strength was approx. 25% above threshold and rectangular pulses of 0.2 ms duration were applied. A single pulse and a 0.8 s train of pulses of a frequency of 28 Hz resulted in a single twitch and a fused tetanic contraction, respectively. Before a series of recordings was started the fibre was stimulated at 3 min intervals to produce at least five isometric tetanic contractions. An interval of 3 min between the contractions was used throughout a series of recordings. The fibre was generally mounted 1-2 h before the experiment began and was periodically stimulated during this time.

Tension recording. A semiconductor strain-gauge transducer (AE 801 Aksjelskapet Mikroelektronikk) was used for recording of tension. A description of this transducer has been given earlier (Edman 1980).

Electromagnetic puller and displacement transducer. Controlled length changes of the fibre were produced by a Dual Servo System, Model 303 (Cambridge Technology). With the device used the releases were terminated within 1 ms. This means that the velocity of the release steps exceeded the velocity of unloaded shortening of the fibre at the temperature used (Edman 1979), thus preventing force production during the shortening phase. The tension recording provided by the servo system of this apparatus was not

utilized in this study. Tension recording was carried out by means of a separate transducer (see above).

Recording and measurement of responses. Tension responses and length changes of the fibre were recorded on a Tektronix 5113 N storage oscilloscope and were photographed on 35 mm orthochromatic film. Measurements from the film records were made in a Nikon model 6C profile projector (10 x magnification) using the stage micrometer readings.

Student's t-test (paired observations) was used for determinations of statistical significance.

RESULTS

A. The influence of varied tonicity of the extracellular medium on the isometric tetanic force production

In agreement with previous findings (eg. April et al. 1968, Edman & Andersson 1968, Edman & Hwang 1977), the isometric tetanic force was found to be reduced after immersing the fibre in hypertonic solution while the reversed effect was obtained in hypotonic medium (Table 1). Thus the tetanic tension value at $2.35 \mu\text{m}$ sarcomere length in 1.22T and 1.44T solutions amounted to $87 \pm 1\%$ and $77 \pm 2\%$, respectively, of the tetanic response in normotonic solution (mean $\pm$ S.E., paired observations). Immersion in 0.81T and 0.62T hypotonic solutions resulted in an enhancement of the contraction response to $110 \pm 2\%$ and $119 \pm 2\%$, respectively (mean $\pm$ S.E., paired observations). The obtained alterations in force production are of the same magnitude as have been observed by Edman & Hwang (1977) and Gulati & Babu (1982).

Table 1. Isometric tetanic force at a sarcomere length of $2.35 \mu\text{m}$ in solutions of varied tonicity, expressed in per cent of the value obtained in ordinary Ringer solution (mean $\pm$ S.E., paired observations).

Relative tonicity	Isometric tetanic force	Significance of the difference, P
0.62T (n=5)	$119 \pm 2 \%$	< 0.001
0.81T (n=7)	$110 \pm 2 \%$	< 0.01
1.22T (n=7)	$87 \pm 1 \%$	< 0.001
1.44T (n=6)	$77 \pm 2 \%$	< 0.001

B. Determination of the depressant effect of active shortening at varied tonicity of the extracellular medium

In order to determine the depressant effect induced by active shortening the approach used was essentially the same as previously described by Edman (1975). Isometric twitch contractions were initiated at a sarcomere length of $2.35\text{ }\mu\text{m}$. A small release, $0.08\text{--}0.11\text{ }\mu\text{m/sarcomere}$ (henceforward called control step), was applied when approximately 75% of the isometric twitch tension had been reached. This control step was just large enough to cause a drop in tension to zero followed by redevelopment of force. A larger (test) release, $0.16\text{--}0.24\text{ }\mu\text{m/sarcomere}$, allowed the fibre to shorten over an additional distance before force redevelopment occurred. The two release steps were carefully timed so that the redevelopment of force was initiated at the same time in both cases. The difference in peak redeveloped force after control and test releases was measured and was expressed in per cent of the isometric tetanic force recorded in each solution at the sarcomere length from which the releases were initiated.

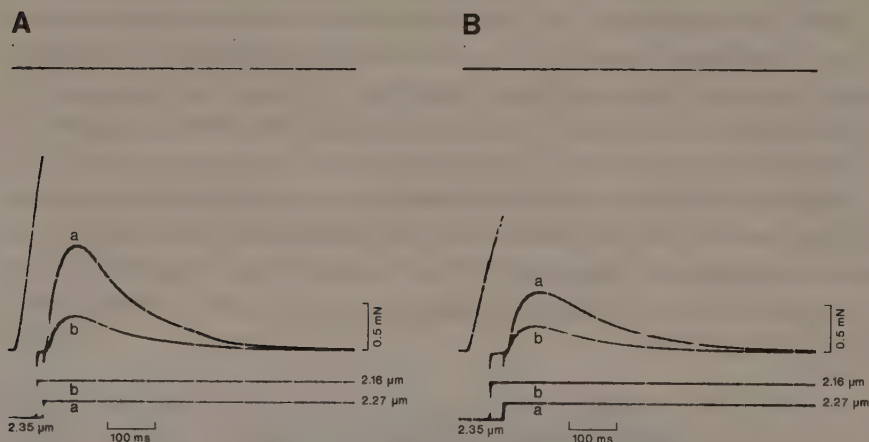


Fig. 1. Influence of sucrose-hypertonic medium on the shortening induced depression. A. Fibre immersed in normotonic solution (ordinary Ringer). B. The same preparation as in A, in 1.22T solution. Isometric tetanic force A: 5.5 mN, B: 4.6 mN. Sarcomere length changes indicated by lower traces. Note that the depressant effect of shortening, i.e. the difference in redeveloped force after the control (a) and the test (b) releases expressed in per cent of the isometric tetanic force, is reduced after immersion of the fibre in the hypertonic solution.

B1. The influence of hypertonicity of the extracellular medium on the depressant effect of active shortening

Fig. 1 and Fig. 2 illustrate recordings of the depressant effect of active shortening obtained in normotonic and hypertonic solutions (1.22T and 1.44T), respectively. The shortening induced depression was found to be steadily reduced as the tonicity of the extracellular medium was raised. Results from altogether 13 fibres are summarized in Table 2. It can be seen that the force depression by shortening decreased from 13.0 (range: 17.8 - 9.4) % in normal Ringer to 7.8 (range: 11.8 - 3.0) % in 1.22T solution. Immersion in 1.44T solution resulted in a reduction of the depressant effect from 19.7% to 6.6% (mean values). The decrease of the shortening effect in the 1.22T solution was thus slightly less than half as large as that caused by the 1.44T solution.

The depressant effect of active shortening has been found to be inversely related to the degree of activation of the contractile system when shortening occurs (Edman 1976, 1980, Ekelund & Edman 1982). It has been suggested that the release of calcium during twitch stimulation is diminished at the hypertonicity used in the present study (Andersson 1973). It can therefore not be excluded that this effect may lead to an underestimation of the hypertonicity induced reduction of the shortening effect.

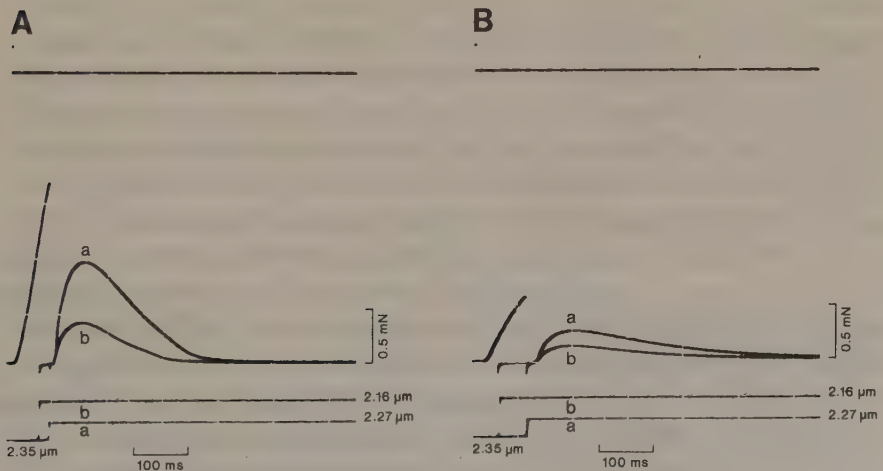


Fig. 2. Recordings illustrating the difference in force depression by shortening in a given preparation immersed in normotonic (A) and 1.44T hypertonic (B) solutions, respectively. Isometric tetanic force A: 3.4 mN, B: 2.7 mN. Lower traces represent sarcomere length changes. For determination of the depressant effect of shortening see Fig. 1.

Table 2. The influence of hypertonicity of the extracellular medium on the depressant effect of active shortening

No. of expt	Force depression (% of isometric tetanic force)			Difference (A-B)	Difference (A-C)
	Normotonic solution (A)	1.22T solution (B)	1.44T solution (C)		
1	12.5	7.6		4.9	
2	9.4	3.0		6.4	
3	11.2	7.5		3.7	
4	15.6	11.0		4.6	
5	17.8	11.8		6.0	
6	9.4	4.0		5.4	
7	15.3	9.9		5.4	
8	15.7		4.6		11.1
9	20.0		5.1		14.9
10	17.8		3.6		14.2
11	23.6		6.2		17.4
12	25.6		15.5		10.1
13	15.3		4.3		11.0
				mean $\pm$ S.E. 5.2 ± 0.3	13.1 ± 1.2
				P < 0.001	< 0.001

B2. The influence of hypotonicity of the extracellular medium on the depressant effect of active shortening

The effect of hypotonicity of the extracellular medium on the depressant effect of active shortening is illustrated in Fig. 3 and Fig. 4. Table 3 summarizes results from 12 experiments. Hypotonic solution was found to enhance the shortening effect. In 7 fibres, the mean force depression by shortening was found to increase from 12.7% to 17.1% by reducing the tonicity from 1.00T to 0.81T. Results obtained from 5 different preparations demonstrated an enhancement of the shortening effect from 17.1% in ordinary Ringer solution to 23.1% in 0.62T solution (mean values).

Fig. 5 illustrates graphically the influence of varied tonicity on the depressant effect of active shortening in three different fibres. In similarity with previous observations (Edman 1980, Ekelund & Edman 1982), the sensitivity to a given amount of shortening varied to some extent from fibre to fibre. It is evident, however, that the degree of force depression was inversely related to the tonicity of the extracellular medium.

C. The influence of tonicity induced changes of fibre diameter on the depressant effect of active shortening

According to measurements made by Edman & Hwang (1977) the cross-sectional area was reduced by ca. 10% in a 1.22T hypertonic solution whereas the

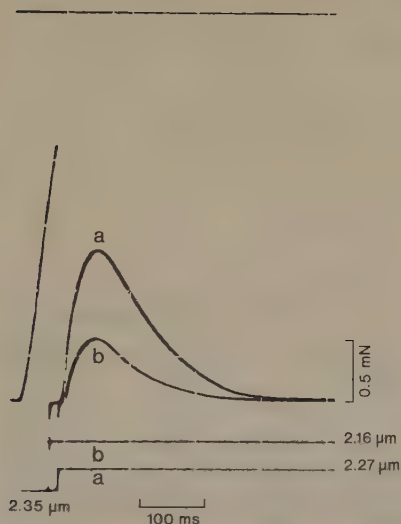
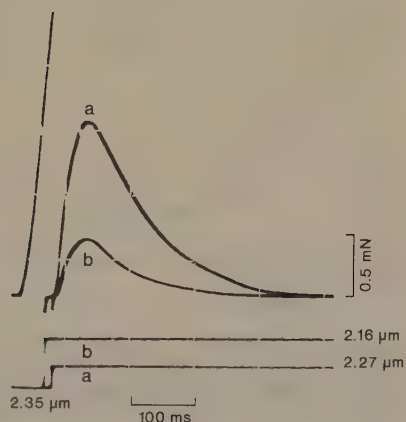
A**B**

Fig. 3. A. Force depression after active shortening obtained in ordinary Ringer solution. B. Force depression, in response to releases of the same amplitude as in A and recorded from the same fibre, in 0.81T solution. Isometric tetanic force A: 5.5 mN, B: 5.9 mN. The changes in sarcomere length are indicated by the lower traces. Measurement of the shortening effect as described in Fig. 1.

cross-sectional area was increased by approximately 10% in a 0.81T hypotonic solution. Since the fibre maintains an almost constant volume as its length is changed (e.g. Elliott et al. 1963, Elliott et al. 1967, Huxley 1969) the differences in fibre diameter that are induced by altered tonicity can be cancelled out by appropriate changes in sarcomere length. This offered a possibility to examine whether the influence of tonicity on the depressant effect of shortening (Section B) was related to the tonicity induced changes in myofilament lattice width.

In the following experiments the depressant effect of shortening was compared in hypertonic and normotonic solutions when the fibre width was maintained constant by adjustment of the sarcomere length. To this end test and control steps were initiated at a sarcomere length of 2.14 μm in 1.22T solution and the shortening induced depression was compared with that recorded at a sarcomere length of 2.35 μm in normal Ringer solution. In both these situations the fibre width can be estimated to be approximately the same. In Table 4 results from 5 different fibres are presented.

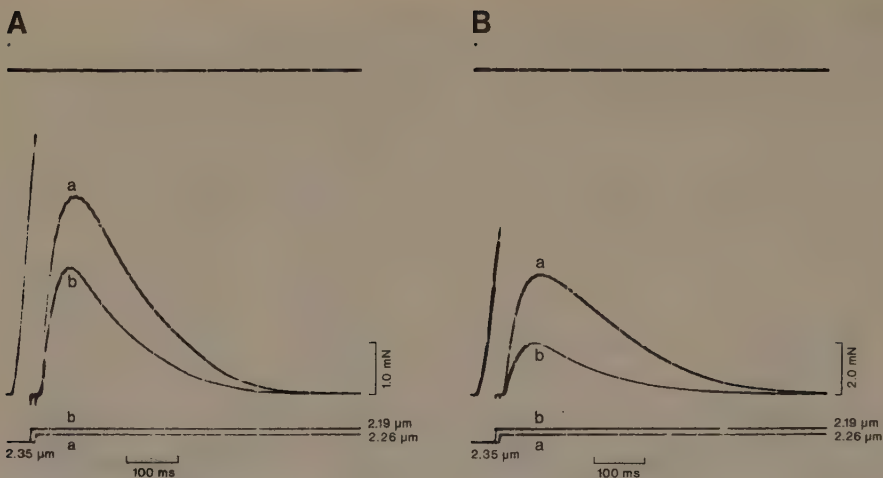


Fig. 4. Force depression in response to a given amount of shortening obtained in the same preparation in 1.00T solution (A) and in 0.62T solution (B), respectively. Isometric tetanic force A: 9.0 mN, B: 11.2 mN. Lower traces demonstrate sarcomere length before and after shortening. The shortening induced depression was measured as described in Fig. 1. Note different tension scales.

The depressant effect of active shortening was found to be 13.3% in the normotonic solution at 2.35 μm sarcomere length and it decreased to 7.3% in the 1.22T solution at 2.14 μm sarcomere length (mean values). The decrease of the shortening effect was thus even slightly more pronounced in this situation, than when hypertonic and normotonic solutions were compared at the same sarcomere length (experiments denoted by the same numeral in Table 2 and Table 4 are from the same fibre). However, it is known that the shortening effect exhibits a length dependence which might influence the results. The shortening induced depression was shown to be maximal at approximately 2.2 μm sarcomere length, while the effect was reduced at shorter and longer lengths (Edman 1976, 1980). The decrease of the shortening effect that was due to a change in sarcomere length, from 2.35 μm to 2.14 μm , was measured in each fibre in normal Ringer and has been taken into account in the 'corrected values' given in Table 4. These corrected data therefore represent an attempt to examine the effect of the tonicity induced decrease in myofilament lattice width per se on the shortening effect. The force depression was calculated to be 13.3% at 2.35 μm sarcomere length in the normal Ringer solution and it decreased to 8.1% at 2.14 μm sarcomere length in the 1.22T solution. This implies that the reduction of the shortening effect was essentially the same as in the situation where the fibre width in the hypertonic and normotonic solutions was different

Table 3. The influence of hypotonicity of the extracellular medium on the depressant effect of active shortening

No. of expt	Force depression (% of isometric tetanic force)			Difference (B-A)	Difference (C-A)
	Normotonic solution (A)	0.81T solution (B)	0.62T solution (C)		
1	12.3	15.9		3.6	
2	15.7	20.7		5.0	
3	8.6	12.8		4.2	
4	12.0	15.9		3.9	
5	13.0	18.0		5.0	
6	17.8	23.4		5.6	
7	9.4	13.0		3.6	
8	20.0		25.2		5.2
9	8.6		13.3		4.7
10	17.8		23.2		5.4
11	23.6		29.9		6.3
12	15.3		23.9		8.6
mean $\pm$ S.E.				4.4 $\pm$ 0.3	6.0 $\pm$ 0.7
P				< 0.001	< 0.001

(compare Table 2 and Table 4). The decrease in myofilament lattice width does therefore not appear to be responsible for the reduction of the depressant effect of shortening that is observed in the hypertonic solution.

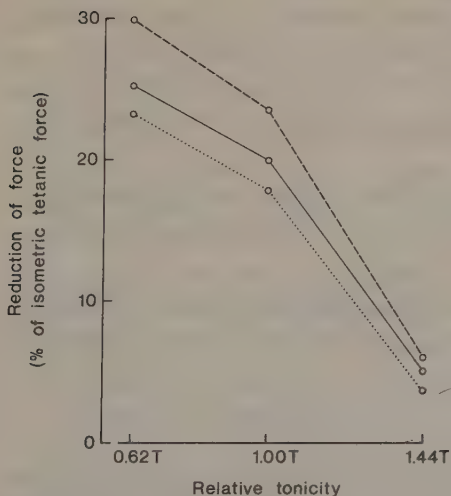


Fig. 5. Depressant effect of active shortening in three different fibres recorded at varied tonicities of the extracellular medium (relative tonicity ranging from 0.62T to 1.44T). Abscissa: Relative tonicity of the extracellular medium. Ordinate: Depressant effect of active shortening in per cent of isometric tetanic force at $2.35\mu\text{m}$ sarcomere length in each solution.

DISCUSSION

The depressant effect of active shortening was found to progressively decrease as the tonicity of the extracellular medium was raised (Table 2). Conversely, hypotonicity of the extracellular medium caused an enhancement of the shortening induced depression (Table 3). In order to evaluate the mechanism underlying these observations it is logical to consider, in the first place, the altered intracellular ionic strength and the variations in fibre width.

The hypertonicity induced decrease in force depression by shortening persisted when the myofilament lattice width was adjusted to be essentially the same as in normal Ringer solution (see further section C under Results). It follows from this, that altered tonicity of the extracellular medium is likely to affect the shortening induced depression by virtue of a change of the intracellular ionic strength. This conclusion is fully in agreement with results obtained from chemically 'skinned' muscle fibres of the frog. These studies demonstrated that the depressant effect of shortening was related to the ionic strength of the surrounding medium. Thus, a diminution of the shortening effect was observed as the ionic strength was increased. Conversely, decreased ionic strength resulted in an enhancement of the shortening induced depression (Ekelund & Edman 1982). The present investigation confirms these findings by suggesting a similar inverse relationship between the intracellular ionic strength and the magnitude of the depressant effect of shortening in the intact muscle fibre.

The mechanism by which a change in ionic strength may affect the cross-bridge kinetics is not yet fully understood. Ionic strength does not appear to have a direct influence on the binding of activator-calcium to troponin (Fuchs 1971). However, it has been demonstrated in several pre-

Table 4. The influence of hypertonicity induced decrease in fibre diameter on the depressant effect of active shortening

No. of expt.	Force depression (% of isometric tetanic force)			Difference (A-B ₂)
	Normotonic solution 2.35 μ m sarcomere length (A)	1.22T solution 2.14 μ m sarcomere length		
		<u>uncorrected (B₁) corrected (B₂)</u>		
1	12.5	6.4	7.9	4.6
2	9.4	2.7	4.6	4.8
3	11.2	5.6	5.7	5.5
4	15.6	10.2	10.2	5.4
5	17.8	11.6	12.0	5.8
Mean $\pm$ S.E.				5.2 $\pm$ 0.2
P				< 0.001

vious investigations that the speed of dissociation of heavy meromyosin from actin increases with increasing ionic strength (Eisenberg & Moos 1970, Rizzino et al. 1970, Greene 1981).

The evidence obtained so far indicates that the shortening induced depression reflects a change in the myofilament system itself. The transitory depression of the contractile activity is presumably due to a reduced affinity of the regulatory troponin-tropomyosin system for activator-calcium (Edman 1975, 1980, Ekelund & Edman 1982).

The influence of ionic strength on the depressant effect of shortening is likely to be elucidated in terms of the kinetic model presented by Adelstein & Eisenberg (1980). With support of biochemical observations (cf. also Bremel & Weber 1972, Weber & Murray 1973), it was stated that cross-bridge cycling has the ability to affect the troponin- Ca^{2+} binding equilibrium. A feedback control was postulated to be exerted from the cross-bridges upon the binding of activator- Ca^{2+} to the regulatory proteins. This implies that an influence upon the actin-myosin-interaction would, indirectly, affect the sensitivity of troponin to calcium and thereby the activation of the contractile system. The effect of ionic strength on the shortening induced depression was explained on the basis of this feedback mechanism (Ekelund & Edman 1982). As the ionic strength is raised, the increased dissociation rate will tend to reduce the number of bridges in 'holding' position. Indirectly, this may alter the troponin-calcium binding so that the decrease in calcium affinity, that is presumed to occur in response to active shortening, is counteracted. Conversely, the shortening induced reduction in affinity of troponin for activator- Ca^{2+} would be augmented as the speed of dissociation of the bridges is slowed down by a decrease in ionic strength.

In conclusion, the present investigation shows that the depressant effect of active shortening is inversely related to the tonicity of the extracellular medium. This effect is not explained by changes in fibre width. In accordance with previously made observations in 'skimmed' fibres, the ionic strength is likely to constitute the modulating factor. The influence of ionic strength on the depressant effect of shortening may be mediated by alterations of the rate of dissociation of cross-bridges. Indirectly, this may affect calcium binding to troponin and thereby the degree of activation of the contractile system.

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THE INFLUENCE OF DANTROLENE, DIAZEPAM, PENTOBARBITAL AND 4-AMINOPYRIDINE
ON SHORTENING INDUCED DEPRESSION IN ISOLATED MUSCLE FIBRES OF THE FROG

M.C. Ekelund

Department of Pharmacology, University of Lund, S- 223 62 Lund, Sweden

The influence of dantrolene, diazepam, pentobarbital and 4-aminopyridine on the depressant effect of active shortening during twitch and tetanus was investigated in isolated muscle fibres of the frog. These drugs are all presumed to alter the kinetics of the release and re-uptake of activator calcium during the excitation-contraction process. The shortening induced depression was calculated as the difference in redeveloped force after a small (control) and a larger (test) release step and was expressed in per cent of the control.

The depressant effect in response to a given amount of shortening during twitch contraction as well as during the plateau of a tetanus was approximately doubled after addition of $10\mu\text{M}$ dantrolene. In contrast, diazepam ($150\mu\text{M}$) reduced the shortening effect during the twitch to about one tenth of the value obtained in ordinary Ringer solution, while the force depression by shortening during tetanic stimulation remained essentially unaltered. Pentobarbital (1.0 mM) reduced the shortening induced depression during the twitch by 95-100%. 4-aminopyridine (3.0 mM) completely abolished the depressant effect of shortening obtained during the twitch and approximately halved the shortening effect during tetanic contraction.

Various previous studies have shown that active shortening affects the contractile behaviour in vertebrate, striated muscle (for references see Edman 1975, 1980, Ekelund & Edman 1982). The observed force depression is transitory and disappears completely during continued stimulation of the fibre (Edman 1975). The collected evidence suggests that shortening during activity has an influence on the myofilament system itself and presumably leads to a reduced affinity of the regulatory troponin-tropomyosin complex for activator calcium (Edman 1980, Ekelund & Edman 1982). This assumption is strengthened by the observation that the shortening induced depression is related to the degree of activation of the contractile system when shortening occurs. A given amount of shortening thus results in a considerably smaller force depression during a fused tetanus than during

a partially fused tetanus or a single twitch (Edman 1976, 1980). Furthermore, the same finding was made in chemically 'skinned' muscle fibres, in which a depressant effect of shortening very similar to that observed in the intact system was obtained (Ekelund & Edman 1982). These studies showed that the shortening induced depression was inversely related to the free calcium concentration of the surrounding medium.

It was considered of interest to investigate how the shortening effect during twitch and tetanus in intact fibres was influenced by various drugs, that are known to affect the contractile behaviour of striated muscle. Experiments were performed to study how the depressant effect of active shortening was affected by diazepam, pentobarbital, 4-aminopyridine and dantrolene, respectively. These four drugs are all presumed to alter the kinetics of activator- Ca^{2+} in the excitation-contraction coupling in different ways. Diazepam, pentobarbital and 4-aminopyridine potentiate the isometric twitch contraction (e.g. DeGroof et al. 1980, Khan 1980, Khan & Edman 1979, 1983 In press). Dantrolene, on the other hand, has the ability to reduce the isometric tension response (e.g. Ellis & Carpenter 1971, Ellis & Bryant 1972, van Winkle 1976, Desmedt & Hainaut 1977, Morgan & Bryant 1977).

METHODS

Preparation and mounting. Single muscle fibres were dissected from the anterior tibialis muscle of Rana temporaria. The course of dissection was similar to that described by Edman & Kiessling (1971). The preparation was transported to the experimental chamber in a Perspex vessel containing Ringer solution (composition, see below). The fibre was mounted horizontally in the muscle bath between hooks of steel wire (0.19 mm thickness) extending from a force transducer and an electromagnetic puller, respectively. This mounting procedure has been described earlier (Ekelund 1983 In press). By adjusting the position of the puller and/or the force transducer the desired rest length of the fibre could be obtained.

Solution. The bathing solution used had the following composition (mM): NaCl 115.5, KCl 2.0, CaCl_2 1.8, Na phosphate buffer 2.0, pH 7.0. Solution was perfused through the muscle chamber throughout the entire experiment (flow rate: approximately 2.8 ml min^{-1}).

Drugs. Dantrolene, pentobarbital and 4-aminopyridine were dissolved in Ringer solution. A stock solution (100 mM) of diazepam was prepared in absolute alcohol from which a 150 μ M solution in Ringer was prepared. The alcohol concentration thereby amounted to 1.5 ml/l Ringer solution. This amount of alcohol did not significantly affect either the isometric force production or the redeveloped force after active shortening.

All chemicals used were of analytical grade and were dissolved in double distilled water.

Dantrolene sodium was obtained from Eaton Chemicals, Norwich, N.Y., USA and diazepam was from Hoffman-La Roche & Co., Basel, Switzerland.

The pH of all solutions was adjusted to 7.0.

Muscle chamber. The experimental chamber (containing 4.5 ml) was milled into a Perspex block and was surrounded by a jacket. A thermostatically controlled water-glycol solution from a Colara Ultrathermostat was circulated through this jacket. The floor of the muscle chamber was made of thin glass. This allowed passage of a laser beam, which was used in order to determine the sarcomere spacing (see further below).

Temperature. The temperature varied between 2.7 and 3.6⁰C from experiment to experiment. However, it was maintained constant to $\pm 0.3^0$ C within any given experiment.

Sarcomere length recording. Laser diffraction technique (helium-neon laser, Spectra Physics, model 133) was used for determination of the sarcomere spacing at rest and during activity (see further Cleworth & Edman 1972). The change in sarcomere length that occurred in response to a release of the fibre during activity was calculated from the change in overall fibre length and the sarcomere length at the onset of the release movement.

Determination of fibre length and cross-sectional area. The distance between the insertions of the fibre to the tendons, i.e. the overall fibre length, was measured with an accuracy of ± 0.05 mm. For this purpose a Zeiss Stereo II microscope was utilized (10 x magnification). The cross-sectional area was calculated by measuring the smallest and largest diameter of the fibre at 40 x magnification, assuming that the cross-section has an elliptical shape.

Stimulation. The preparation was stimulated by passing current between two platinum plate electrodes, placed on either side of the fibre. Rectangular pulses (0.2 ms duration) were used and the stimulation strength

was adjusted to be approximately 25% above threshold. A single pulse or a 1 s train of pulses with a frequency of 28 Hz resulted in a single twitch and a fused tetanus, respectively. In order to produce a tetanic contraction in the presence of 150 μ m diazepam it was necessary to reduce the stimulation frequency to 10-15 Hz. A 3-min interval between the contractions was maintained throughout the entire experiment. The fibre was mounted approximately 1-2 h before the actual experiment started. During this time it was periodically stimulated. This period ended by five or more isometric tetanic contractions induced at 3-min intervals.

Tension recording. Tension was recorded by means of a semiconductor strain-gauge transducer (AE 801, Aksjeselskapet Mikroelektronikk). This transducer has been described previously (Edman 1980).

Electromagnetic puller and displacement transducer. A Dual Servo System, Model 303 (Cambridge Technology) was utilized in order to produce release steps of desired amplitude. The releases were fast enough to prevent any force production during the shortening phase (rise time appr. 1-2 ms). Tension recording was not obtained from the servo system of this apparatus. For this purpose a separate semiconductor strain-gauge transducer was used (see above).

Recording and measurement of responses. Tension and length changes were displayed on a Tektronix 5113N storage oscilloscope and were photographed on 35 mm orthochromatic film. The measurements from the film records were carried out in a Nikon model 6C profile projector, using the stage micrometer readings.

RESULTS

A. Determination of the depressant effect of shortening during activity

The method used for determination of the shortening induced depression was similar to that previously described (Edman 1975). Twitch contractions were initiated at 2.26 μ m sarcomere length. During the rising phase of the contraction, at approximately 75% of the isometric twitch tension value, a small release step was applied. This release (the control step) had an amplitude of 0.07 μ m/sarcomere and was just large enough to cause a complete drop in tension, followed by immediate redevelopment of force. A larger release step (the test step), 0.15 μ m/sarcomere or 0.28 μ m/sarco-

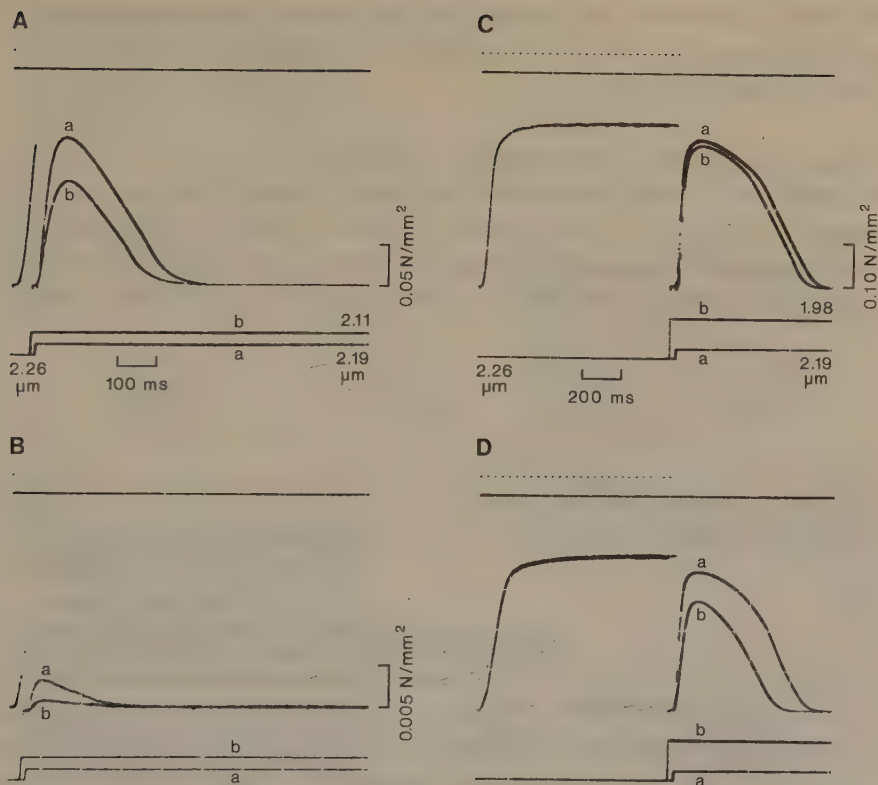


Fig. 1. The influence of dantrolene on the depressant effect of active shortening during twitch and tetanus. A & C: Fibre immersed in normal Ringer. B & D: After addition of $10 \mu\text{M}$ dantrolene. Note that the shortening effect, i.e. the difference in redeveloped force after the control (a) and test (b) releases in per cent of the control, is increased after addition of dantrolene. Lower traces indicate the sarcomere length before and after shortening.

mere, permitted the fibre to shorten over an additional distance before force redevelopment was initiated.

Tetanic contractions were initiated at the same sarcomere length, i.e. at $2.26 \mu\text{m}$. The control step, $0.07 \mu\text{m/sarcomere}$, occurred previous to the last stimulus. The test release used had an amplitude of $0.28 \mu\text{m/sarcomere}$.

The force redevelopment after both control and test releases occurred at a sarcomere length between $2.19 \mu\text{m}$ and $1.98 \mu\text{m}$, i.e. within the plateau of the length-tension relation (e.g. Gordon et al. 1966, Edman 1979). The control and test steps were carefully timed so that force redevelopment was initiated at the same moment in both cases. The difference in peak

redeveloped force after the control and test release steps was measured and was expressed in per cent of the control (i.e. the force redeveloped after the small shortening).

B. Active shortening in the presence of dantrolene

Dantrolene has a direct reductive effect on the isometric contractile response in vertebrate striated muscle, presumably due to inhibition of calcium release from the sarcoplasmic reticulum in response to membrane excitation (van Winkle 1976, Desmedt & Hainaut 1977, Morgan & Bryant 1977).

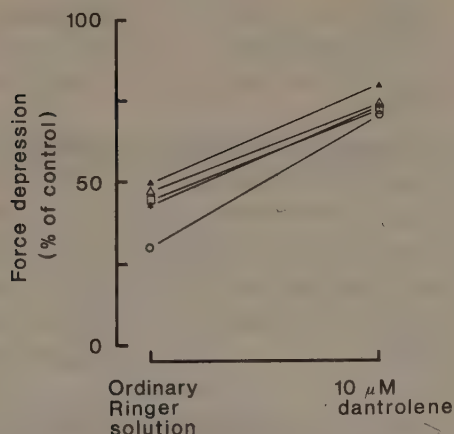


Fig. 2. The shortening induced depression during twitch contraction in five different fibres. The lines join the values obtained in normal Ringer and after addition of 10 μ M dantrolene, respectively. The depressant effect was measured as the difference in peak redeveloped force after a small (control) and a large (test) release and was expressed in per cent of the control.

This effect leads to a marked reduction of the isometric twitch response, while the tetanic tension level is only little affected (Putney Jr. & Bianchi 1973, 1974). In the present study, 10 μ M dantrolene reduced the isometric twitch tension by approximately 97% (range: 95.5–97.9), while the decrease of the tetanic response amounted to approximately 7% (range: 5.3–9.3).

The influence of dantrolene on the depressant effect of active shortening during twitch contraction is illustrated in Fig. 1 A, B. It can be seen that addition of dantrolene resulted in an enhancement of the shortening effect. Fig. 2 summarizes the effect of dantrolene on the shortening induced depression obtained during twitch contraction in five different fibres. The force depression of 0.08 μ m/sarcomere shortening (in excess of control) amounted to 42.5% in normal Ringer and it increased to 72.8% in the presence of 10 μ M dantrolene (mean values). Force depression by shortening performed during the plateau of a fused tetanic contraction was affected in a similar way, as is illustrated in Fig. 1 C, D and in

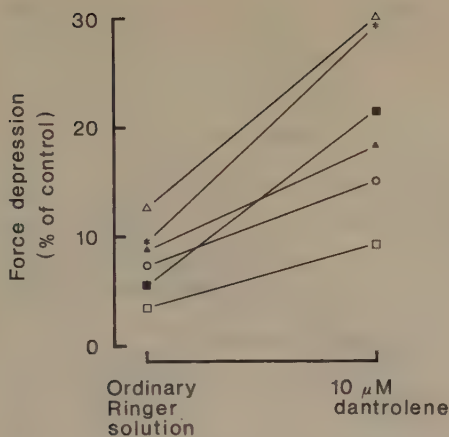


Fig. 3. The depressant effect of active shortening during tetanus in six different preparations. Results obtained in ordinary Ringer solution and after addition of $10\mu\text{M}$ dantrolene, respectively, are joined by a given line. For calculation of the shortening effect, see Fig. 2.

Fig. 3. The depressant effect of $0.21\mu\text{m/sarcomere}$ shortening (in excess of control) increased from 7.9% to 20.7% after addition of dantrolene ($n=6$, mean values).

C. Active shortening in the presence of diazepam

Diazepam has a pronounced potentiating effect on the isometric twitch response in vertebrate striated muscle. The underlying mechanism can only partly be attributed to a prolongation of the action potential duration. Diazepam appears to exert a more specific effect on the mechanism of release of activator- Ca^{2+} . It has been proposed that diazepam may influence the movement of charge within the plasma membrane that is supposed to be involved in the coupling between membrane excitation and release of activator calcium (Khan & Edman 1983 In press, see also Schneider & Chandler 1973, Hui 1982). The potentiating effect was found to be optimal at a concentration of approximately $150\mu\text{M}$ (Khan & Edman 1983 In press). In the present investigation $150\mu\text{M}$ diazepam resulted in an increase of the isometric twitch tension that amounted to 94.4% (range: 90.2-97.2) of the isometric tetanic force, while the tetanus response was virtually unaltered.

Fig. 4 A-C illustrates that the depressant effect of active shortening during a twitch was markedly reduced in the presence of diazepam and that this effect was reversible. In Fig. 5 results from 6 different fibres are summarized. Shortening of $0.08\mu\text{m/sarcomere}$ (in excess of control) resulted in a force depression that amounted to 50.6% in ordinary Ringer solution and it was reduced to 3.8% after addition of diazepam. The shortening in-

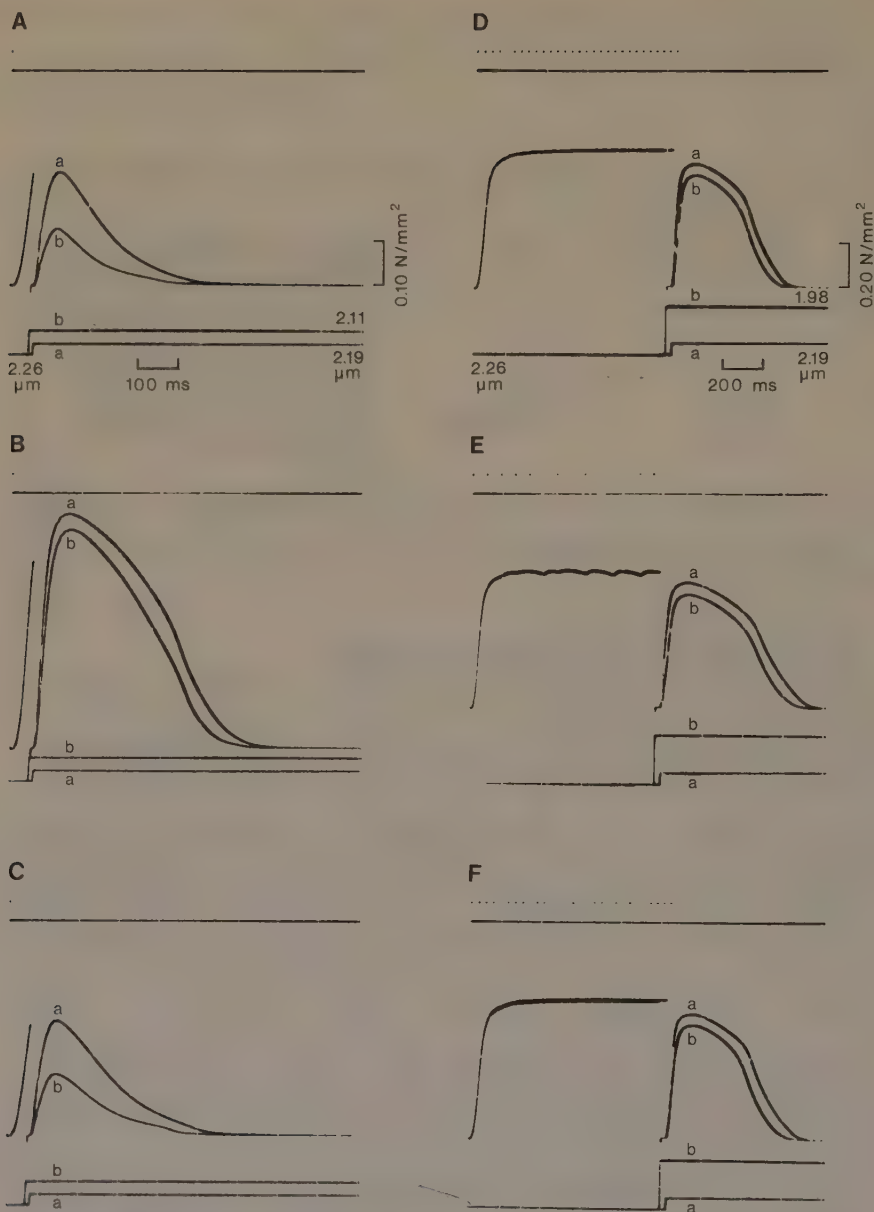
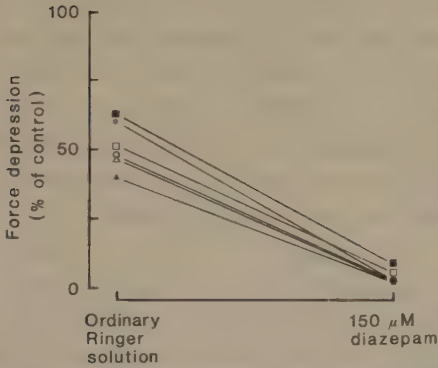


Fig. 4. The effect of diazepam on the shortening induced depression during twitch and tetanic contraction. A & D: Fibre immersed in ordinary Ringer. B & E: After addition of 150 μM diazepam. C & F: Reimmersion in normal Ringer. Measurement of the shortening effect as described in Fig. 1. Sarcomere length changes indicated by the lower traces. Note that addition of diazepam results in a reversible reduction of the shortening effect obtained during twitch contraction, while the effect of active shortening during tetanus is essentially unaltered.

A



B

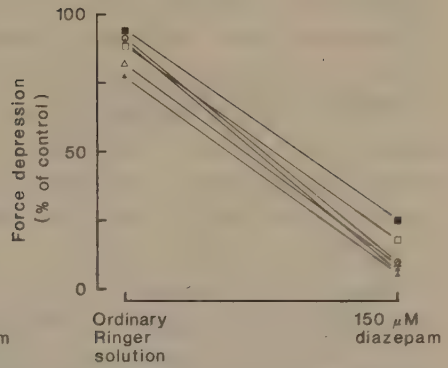


Fig. 5. The depressant effect of active shortening during twitch contraction before and after addition of $150\mu\text{M}$ diazepam. A: The depressant effect of $0.08\mu\text{m/sarcomere}$ shortening (in excess of control). B: Force depression in response to a larger amount of shortening, $0.21\mu\text{m/sarcomere}$ (in excess of control). An identical symbol in A and B denotes the same fibre. The shortening induced depression was measured as described in Fig. 2.

duced depression in response to a larger amount of shortening, $0.21\mu\text{m/sarcomere}$ (in excess of control), was found to decrease from 87.7% to 12.3% (mean values). Force depression by shortening performed during the plateau of a tetanus appeared to be essentially unaffected by the application of diazepam, as can be observed in Fig. 4 D-F. Fig. 6 summarizes results from similar experiments obtained from 6 different fibres. The shortening effect during tetanic contraction in the presence of $150\mu\text{M}$ diazepam; 6.8%, was not significantly different from that obtained in normal Ringer solution; 6.6% (mean values).

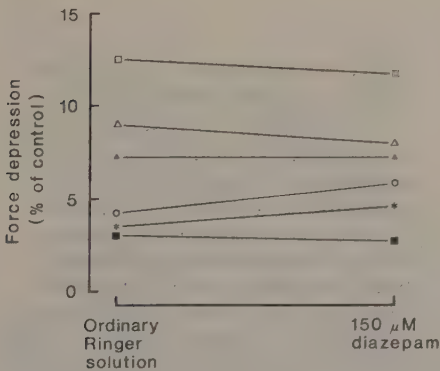
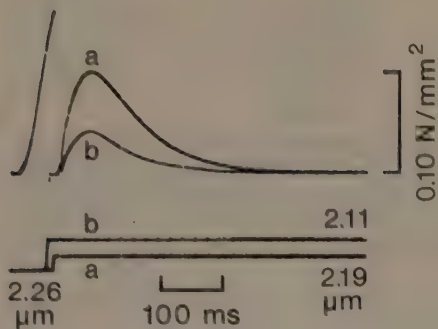


Fig. 6. The depressant effect of active shortening during tetanic contraction in six different preparations. The lines connect the magnitude of the shortening effect in normal Ringer solution and after immersion of the fibre in Ringer solution containing $150\mu\text{M}$ diazepam. The shortening induced depression was calculated as described in Fig. 2.

A



B

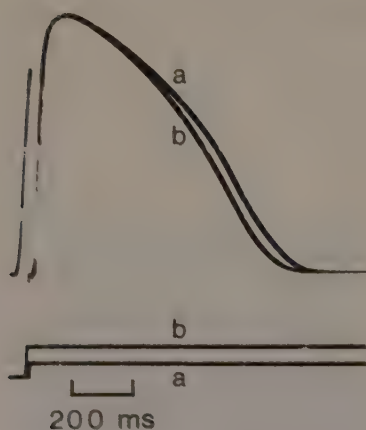


Fig. 7. The depressant effect of active shortening during twitch contraction in normal Ringer (A) and after addition of 1.0 mM pentobarbital (B). Note that the shortening induced depression, i.e., the difference in peak redeveloped force after the control (a) and test (b) releases, is eliminated in the presence of 1.0 mM pentobarbital. Sarcomere length changes in response to shortening indicated by lower traces.

D. Active shortening in the presence of pentobarbital

Pentobarbital is known to exert a direct potentiating effect on muscle contraction (Foulks et al. 1973, Khan 1980, also for further references). This has been suggested to be the result of three different mechanisms; an increased rate of release of calcium, an increased duration of the action potential and an inhibition of calcium resequestration (Khan 1980). Maximal potentiation was considered to be obtained with 1.0 mM pentobarbital (Khan 1980). This concentration increased the isometric twitch response to 90.7% (range: 87.3-94.8) of the tetanic tension, in the present investigation.

The depressant effect in response to $0.08 \mu\text{m/sarcomere}$ shortening (in excess of control) during twitch contraction disappeared completely after the addition of 1.0 mM pentobarbital as is illustrated in Fig. 7 A, B. Fig. 8 summarizes results from similar experiments. The depressant effect of $0.08 \mu\text{m/sarcomere}$ shortening (in excess of control) resulted in a force depression that amounted to 42.1% ($n=5$, mean values) in ordinary Ringer. Pentobarbital had the ability to completely eliminate this depressant effect in all fibres. A larger amount of shortening, $0.21 \mu\text{m/sarcomere}$ (in excess of control), caused a more pronounced shortening induced depression; 79.4% in normal Ringer. This effect was reduced to 1.8% ($n=5$, mean values) after addition of 1.0 mM pentobarbital. Neither the twitch potentiation

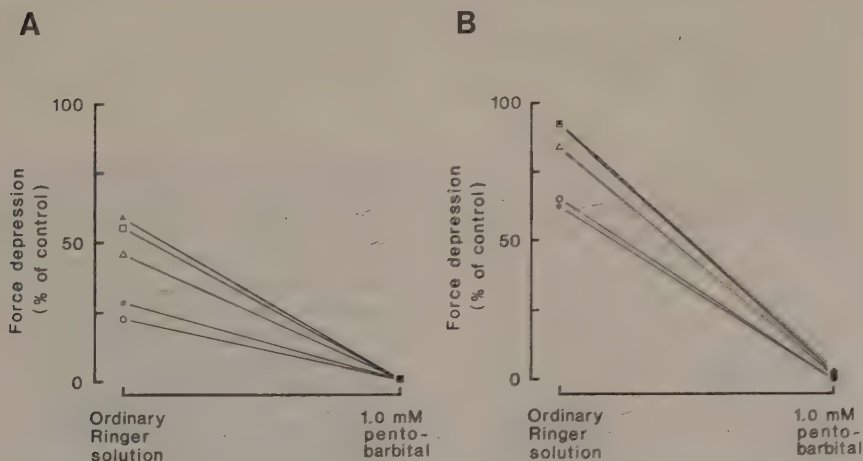


Fig. 8. The depressant effect of $0.08 \mu\text{m/sarcomere}$ (A) and $0.21 \mu\text{m/sarcomere}$ (B) shortening (in excess of control) during twitch contraction. Each line joins the value obtained in normal Ringer solution and after addition of 1.0 mM pentobarbital, respectively. Results from five different fibres are given. An identical symbol in A and B denotes the same fibre. For calculation of the shortening effect, see Fig. 2.

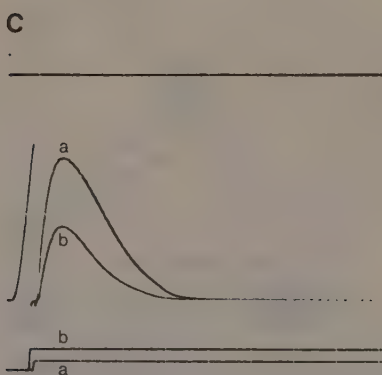
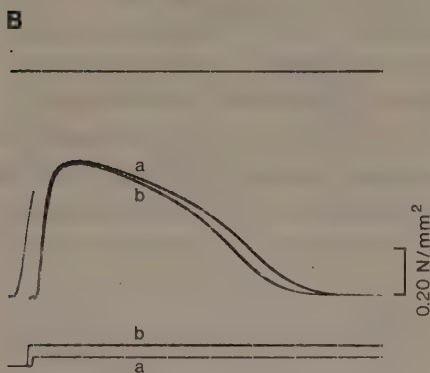
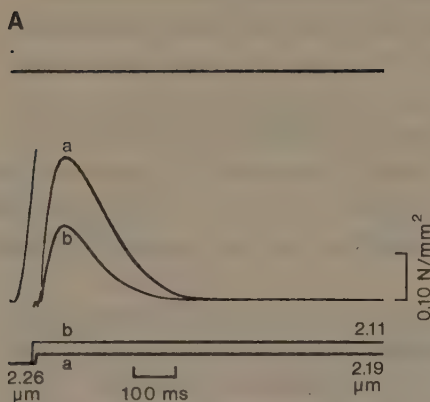


Fig. 9. A: The depressant effect of active shortening during twitch contraction in ordinary Ringer solution. B: After addition of 0.5 mM pentobarbital. C: Reimmersion in ordinary Ringer. Measurement of the depressant effect by shortening performed as in Fig. 1. Note that the shortening induced depression is markedly diminished in the presence of 0.5 mM pentobarbital and that this effect is reversible after re-immersion in normal Ringer. The changes in sarcomere length are indicated by the lower traces.

nor the reduction of the shortening effect was completely reversible. Pentobarbital in a concentration of 0.5 mM resulted in a slightly less pronounced reduction of the depressant effect of shortening. The effect of 0.5 mM pentobarbital was fully reversible (Fig. 9 A-C).

E. Active shortening in the presence of 4-aminopyridine

4-aminopyridine (4-AP) enhances the transmitter release at the neuromuscular junction (Molgo et al. 1977, Lundh & Thesleff 1977) but has been shown to potentiate the twitch response by a direct action on the muscle fibre (Khan & Edman 1979). The observed twitch enhancement was interpreted to be mainly due to an increased duration of the action potential. 3.0 mM 4-aminopyridine was found to produce maximum potentiation of the isometric twitch contraction response, while the tetanus remained essentially unaffected (Khan & Edman 1979). This concentration was used in the present study and resulted in a twitch potentiation of 97.5% (range: 94.8-99.3) of the isometric tetanus. The tetanic tension response was not altered by addition of 4-aminopyridine.

Fig. 10 summarizes the reductive effect of 4-aminopyridine on the shortening induced depression during a twitch. In ordinary Ringer, the depressant effect of 0.08 $\mu\text{m/sarcomere}$ and 0.21 $\mu\text{m/sarcomere}$ shortening (in excess of control) was 54.7% and 87.2%, respectively ($n=5$, mean values).

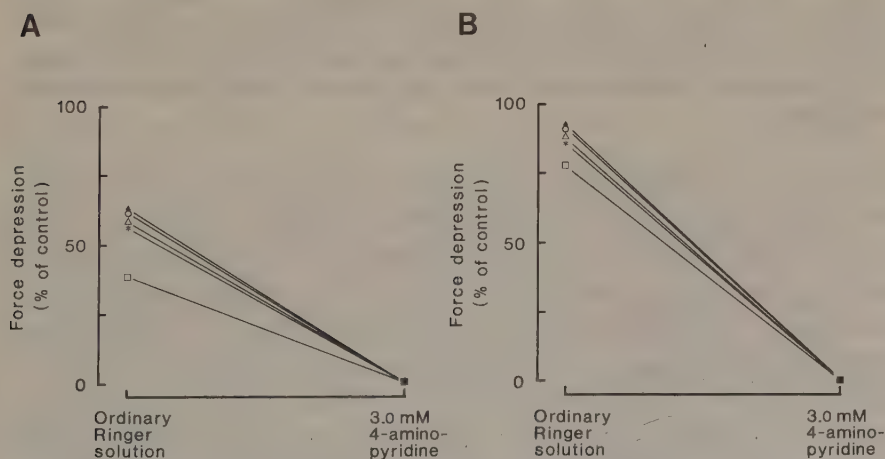


Fig. 10. The depressant effect of shortening applied during twitch contraction in ordinary Ringer and in the presence of 3.0 mM 4-aminopyridine, respectively. The lines connect the values obtained in these two situations. The amplitude of shortening (in excess of control) was 0.08 $\mu\text{m/sarcomere}$ in (A) and 0.21 $\mu\text{m/sarcomere}$ in (B). An identical symbol in A and B denotes the same fibre. Measurement of the shortening induced depression performed as in Fig. 2.

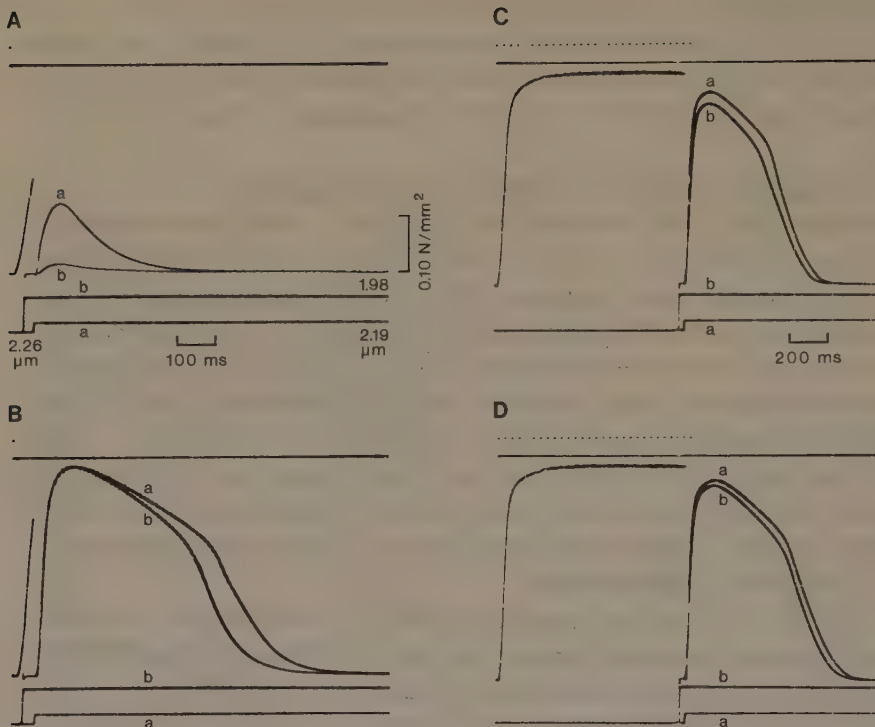


Fig. 11. Recordings illustrating the influence of 4-aminopyridine on the depressant effect of shortening during twitch and tetanus. A & C: Fibre immersed in ordinary Ringer solution. B & D: In the presence of 3.0 mM 4-aminopyridine. Note that the shortening induced depression is reduced during tetanus and is completely abolished during twitch contraction. The shortening effect measured as described in Fig. 1. Lower traces represent sarcomere length changes.

Addition of 3 mM 4-aminopyridine completely eliminated the shortening induced depression. Fig. 11 A, B demonstrates recordings of the entire abolition of the depressant effect of shortening during twitch contraction after addition of 4-aminopyridine. The shortening effect obtained during the plateau of a tetanus was affected in the same direction, but was not completely abolished (Fig. 11 C, D). In Fig. 12 results from six different fibres are given. The depressant effect in response to $0.21 \mu\text{m/sarcomere}$ shortening (in excess of control) during tetanus was 5.2% in normal Ringer and it was reduced to 2.8% after application of 3.0 mM 4-aminopyridine (mean values). The twitch potentiating effect of 4-aminopyridine was not completely reversible (see also Khan & Edman 1979), nor was the reduction of the shortening induced depression.

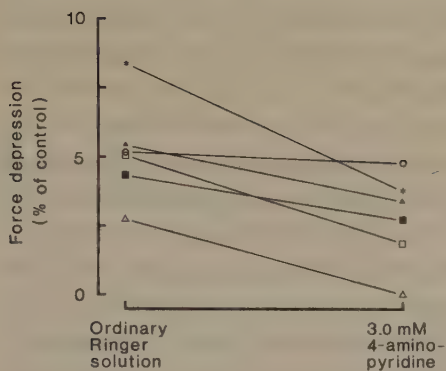


Fig. 12. Shortening induced depression in six different fibres during tetanic contraction. The lines connect values obtained in ordinary Ringer solution and after addition of 3.0 mM 4-amino-pyridine, respectively. The depressant effect of active shortening was calculated as described in Fig. 2.

DISCUSSION

The shortening effect during twitch contraction

All previous investigations indicate that the depressant effect of active shortening is the result of a change on the myofilament level (Edman 1975, 1980, Ekelund & Edman 1982, Ekelund 1983 In press). The shortening effect is suggested to be due to a transitory reduction in affinity of the regulatory troponin-tropomyosin complex for activator calcium. In the skinned fibre preparation the magnitude of the depressant effect in response to a given amount of shortening was found to be critically dependent on the calcium ion concentration in the surrounding medium. An increase of the amount of free calcium caused a progressive reduction of the shortening effect, in some fibres leading to an almost complete disappearance of the depressant effect (Ekelund & Edman 1982). The present study provided an opportunity to investigate the relationship between the magnitude of the shortening effect and the amount of activator calcium in the myofibrillar space in the intact system. For this purpose the influence of diazepam, pentobarbital, 4-aminopyridine and dantrolene on the depressant effect of active shortening was studied during twitch and tetanus in single muscle fibres of the frog.

Addition of diazepam, pentobarbital or 4-aminopyridine resulted in a marked reduction or, in several cases, a complete abolition of the shortening effect during twitch contraction. These three substances, acting via different mechanisms, have a twitch potentiating effect. They are all presumed to raise the concentration of calcium in the myofibrillar space

in response to membrane excitation (e.g. Foulks et al. 1973, DeGroof et al. 1980, Khan 1980, Khan & Edman 1979, 1983 In press). The present results would seem to indicate that an appropriate increase of the amount of activator calcium completely eliminates the shortening induced depression in the intact muscle fibre.

The capacity of various twitch potentiating agents to reduce the shortening effect does not appear to be strictly related to the magnitude of the force enhancement. In the present study, the maximum twitch potentiating effect of diazepam was close to that obtained by 4-aminopyridine and was higher than that induced by pentobarbital. However, the capability of diazepam to reduce the depressant effect of active shortening during twitch contraction was appreciably lower than that produced by either pentobarbital or 4-aminopyridine (compare Fig. 5 with Fig. 8 and Fig. 10). Although the mechanism underlying the twitch potentiation of diazepam appears to be different from that of pentobarbital and 4-aminopyridine (see further above), there is no obvious explanation of the observed difference in capacity of the various substances to affect the shortening induced depression. Nevertheless, it can safely be concluded that twitch potentiating drugs greatly reduce the depressant effect of active shortening.

The results obtained in the presence of dantrolene provide additional evidence that there is a close connection between the intracellular calcium concentration and the magnitude of the depressant effect of active shortening. The addition of this agent resulted in an enhancement of the shortening induced depression. Dantrolene is presumed to reduce the isometric tension response by virtue of decreasing the release of activator calcium in response to membrane excitation (e.g. Ellis & Carpenter 1971, van Winkle 1976, Desmedt & Hainaut 1977, Morgan & Bryant 1977). Augmentation of the shortening effect by an intervention that is thought to decrease the myofibrillar calcium is fully in line with the assumption that active shortening reduces the affinity of troponin for activator- Ca^{2+} .

The depressant effect of active shortening during tetanus

4-aminopyridine was found to reduce the depressant effect of a given amount of shortening during the plateau of a tetanus. However, in contrast to the effect obtained during twitch contraction, the shortening induced depression could not be completely eliminated. The isometric tetanic tension in the presence of dantrolene reached a slightly higher level than attained during a potentiated twitch after addition of pentobarbital. However, the

depressant effect in response to a given amount of shortening was considerably smaller in the latter situation. These findings suggest that the contractile system is more sensitive to active shortening during tetanus in both these cases. This is an interesting observation as it would seem to indicate that the magnitude of the depressant effect after a given amount of shortening is not solely governed by the degree of activation of the contractile system, i.e. by the amount of calcium bound to troponin, when shortening occurs. A possibility worth considering is that the sensitivity to active shortening is greater and the ability to neutralize the shortening effect less pronounced after steady state force production has been attained than during the development of contractile activity. This may explain the findings referred to above and the fact that the depressant effect of a given amount of shortening in ordinary Ringer solution is smaller during a tetanus than during a twitch. Calcium is presumed to be present in excess in the myofibrillar space during tetanic contraction in normal Ringer solution (Edman & Flitney 1982, see also Hellam & Podolsky 1969, Julian 1971, Blinks et al. 1978, Lopez et al. 1981, Taylor et al. 1982). This would to some extent compensate for a greater sensitivity to active shortening but would not be sufficient to entirely eliminate the shortening effect. Furthermore, it would explain the finding that the depressant effect of active shortening was considerably lower during a potentiated twitch than during a tetanus that was reduced in amplitude by dantrolene. In this case less calcium would be available during the tetanus to neutralize the shortening induced depression.

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Depressant effect of active shortening in the anterior byssus retractor muscle of *Mytilus edulis*

M. C. EKElund

Department of Pharmacology, University of Lund, S-223 62 Lund, Sweden

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The effect of shortening during activity, previously characterized in vertebrate striated muscle, was investigated in the anterior byssus retractor muscle (ABRM) of the mollusc *Mytilus edulis*. This muscle is considered to have an essentially myosin-linked Ca^{2+} -regulatory system. Release steps of different amplitude were performed during isometric phasic contraction, and force redevelopment was recorded at a muscle length L_1 , defined as 90% of the muscle length at which a slight resting tension, approximately 1 mN, appeared in the presence of 2.5×10^{-5} M 5-HT. Active shortening caused a graded depression of the contractile force without affecting the total duration of the mechanical response. Peak redeveloped force after muscle shortening of 0.06 L_1 and 0.18 L_1 was reduced by approximately 1.5% and 7.0%, respectively, of the isometric tension value at L_1 . The shortening effect was fully reversible, and had a lifetime of approximately 8 to 9 s. The depressant effect of active shortening was augmented at a reduced degree of activation of the muscle. The presence of caffeine and dantrolene and altered tonicity of the extracellular medium (0.9 T–1.2 T) did not significantly affect the shortening induced depression obtained at maximum phasic activation of the preparation. The nature of the shortening effect is compared to that obtained in vertebrate striated muscle and is discussed on the basis of differences in Ca^{2+} -regulation of the contractile system in these two muscles.

Key words: Mollusc muscle, anterior byssus retractor muscle (ABRM), phasic contraction, active shortening, force depression, caffeine, dantrolene, tonicity

Several investigations have indicated that muscle shortening during activity has an influence upon the contractile behaviour. This has been observed in amphibian and mammalian cardiac muscle (Brady 1966, Edman & Nilsson 1971, Bozler 1972, Kaufmann et al. 1972, Bodem & Sonnenblick 1974, Reggiani et al. 1980) and vertebrate skeletal muscle (Jewell & Wilkie 1960, Edman & Kiessling 1971, Briden & Alpert 1972, Dickinson & Woledge 1973). The effect has been noted in whole muscle *in situ* in the body (Joyce & Rack 1969, Joyce et al. 1969) as well as in isolated single muscle fibres (Edman & Kiessling 1971, Edman 1975, 1980) and appears to reflect a physiological behaviour in response to active shortening. More detailed studies to investigate the nature of the mechanism underlying the depressant effect of active shortening have been

performed in intact (Edman 1975, 1980) and chemically 'skinned' single muscle fibres of the frog (Ekelund & Edman 1982). These investigations have excluded a number of possible causes of the depressant effect of active shortening, such as sarcomere non-uniformity, depletion of chemical energy, altered kinetics of the release and reuptake of activator calcium during the excitation-contraction coupling and a change in series elastic compliance. The experimental evidence suggests that active shortening leads to a temporary deactivation of the contractile system, an effect which is fully restored during continued isometric activity.

Previous studies have indicated that active shortening in vertebrate striated muscle has a direct effect on the contractile system, presumably leading to a reduced affinity of the regulatory troponin-

tropomyosin complex for activator- Ca^{2+} (Edman 1980, Ekelund & Edman 1982). With this in view, it was of interest to extend the investigation of this phenomenon to include a muscle with a different Ca^{2+} -regulatory mechanism. For this purpose the anterior byssus retractor muscle (ABRM) of the mollusc *Mytilus edulis* was chosen. This muscle is considered to be mainly, if not entirely, regulated by the myosin and associated light chains (Kendrick-Jones et al. 1970, Szent-Györgyi et al. 1973, Lehman & Szent-Györgyi 1975, Goldberg & Lehman 1978, Lehman 1981, for review see Kendrick-Jones & Scholey 1981). In the present investigation experiments have also been performed in order to find out if the effect of shortening during activity is dependent on the degree of activation of the contractile system. Furthermore, the effect of active shortening at varied tonicity of the extracellular medium is reported.

METHODS

Preparation and mounting. The anterior byssus retractor muscle (ABRM) of the mollusc *Mytilus edulis* was studied. The specimens were obtained during wintertime from the Marine Biological Station, Fiskebäckskil, Sweden. They were kept in aerated sea water at 4°C and used within five to six weeks. The animal was opened by cutting the adductor muscles and the ABRM (in situ length, approximately 4 to 5 mm) was dissected free in sea water. A thread of silk was tied behind the muscle's insertion into the byssal organ and a small piece of shell was left behind at the other end of the muscle. The preparation was thereafter transported in a small Perspex vessel containing sea water to the experimental chamber. The muscle was mounted horizontally in a jacketed Perspex bath (5 ml) containing artificial sea water (composition, see below). The silk thread was attached to the force transducer and the piece of shell was fastened to a clip that was attached to an arm extending from the electromagnetic puller. The preparation was stretched to its in situ length and was kept resting in the bath for approximately 1 h before the actual experiment started. The muscle chamber was continuously perfused with oxygenated artificial sea water during mounting and throughout the experiment.

Solutions. An artificial sea water solution of the following composition was used (mM): NaCl 200, KCl 10, CaCl_2 20, MgCl_2 54, PIPES [Piperazine-N,N'-bis(2-ethane Sulfonic Acid)] 10, pH 7.5. To the solutions used under section D (Results) dantrolene sodium (15 μM) or caffeine (0.12–0.50 mM) was added to the artificial sea water. The solutions used under section E were made hypertonic by addition of 66.7 mM or 133.0 mM sucrose or hypotonic by reduction of NaCl from 200 mM to 165 mM. Assuming the osmotic coefficient of NaCl to be 0.93 and using the formula provided by Dydyńska & Wilkie

(1963) for calculation of the osmotic coefficient of sucrose, the relative tonicity of the hypertonic solutions amounted to 1.1 T and 1.2 T, respectively, of the normotonic (T) solution. A reduction of the NaCl concentration to 165 mM resulted in a relative tonicity of 0.9 T. It proved difficult to further reduce the NaCl concentration since this led to a diminished contraction response, possibly reflecting incomplete activation of the muscle under these circumstances. In order to abolish apparent resting tension and to increase the relaxation rate $2.5 \times 10^{-5} \text{ M } 5\text{-HT}$ was added to each solution used during the actual experiment (Lowy & Millman 1963). The solutions were constantly oxygenated and perfused through the muscle chamber at a speed of approximately 4 ml min^{-1} . Before any recordings were carried out in experiments performed under sections D and E under Results, the muscle was immersed for at least 30 min in the new solution.

Determination of muscle length and cross-sectional area. The overall muscle length was measured as the distance between the muscle's attachment to the shell and its insertion into the byssal organ. A Zeiss Stereo II microscope ($\times 16$ magnification) was used for this purpose and the distance was measured to the nearest 0.03 mm. The cross-sectional area was calculated by measuring the smallest and largest diameter of the muscle to the nearest 0.010 mm in a Zeiss Stereo II microscope ($\times 40$ magnification) under the assumption that the cross-section had an elliptical shape.

Temperature. The temperature was controlled by circulating a water-glycol mixture from a Colora Ultrathermostat through a jacket surrounding the muscle chamber. The temperature was kept constant to $\pm 0.3^{\circ}\text{C}$ during any given experiment and varied between 10.3 and 11.2°C between the different experiments.

Stimulation. Alternating current (50 Hz) was applied through two platinum plate electrodes, placed symmetrically on either side of the muscle, in order to obtain isometric phasic contractions. The field strength was 1.7 V mm^{-1} and the stimulation period 8 s. This provided optimal phasic activation of all preparations used. In some experiments described under section B and C under Results the stimulation period was reduced to 1.3–1.5 s. An interval of 5 min was used between the stimulation periods during the entire experiment.

Tension recording. A semiconductor strain-gauge transducer (AE 801, Aksjeselskapet Mikroelektronikk) was used as previously described (Edman 1980). The transducer gave a linear response up to at least 10 g.

Electromagnetic puller, servo system and displacement transducer. The system utilized was essentially the same as previously described (Edman 1975). Release steps of different amplitude were produced, the speed of release (rise time approximately 5 ms) being sufficiently high to prevent any force production during the shortening phase. The same final muscle length was attained after the different steps by adjusting the rest length of the muscle (see further below). This was done with an accuracy of 12 μm , i.e. 0.3% of the in situ muscle length.

Recording and measurement of responses. Tension and length changes were displayed on a Tektronix 5113N storage oscilloscope and photographed on 35 mm orthochromatic film. Measurements were made in a Nikon

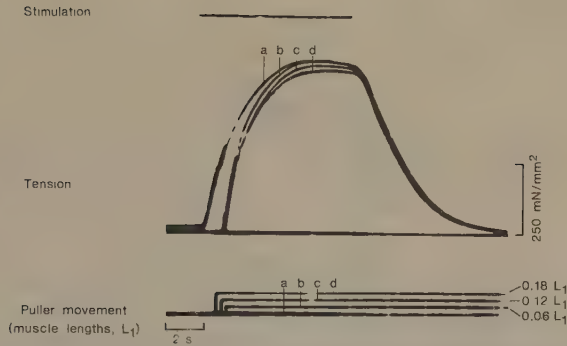


Fig. 1. Redevelopment of tension after different amounts of active shortening during isometric phasic contraction in the ABRM. The contractions were initiated at different muscle lengths (adjusted at the force transducer end) so as to allow the muscle to redevelop force at the same length (L_1 , for definition, see text) after each release step. The release movements were timed so that this force redevelopment was initiated at the same time in each case. Myogram *a* represents the isometric tension at the muscle length L_1 . The depressant effect, i.e. the decrease in peak redeveloped force after test releases (*c* and *d*) relative to peak force after control release (*b*), was found to progressively increase by the amount of shortening.

model 6C profile projector at $10\times$ magnification using the stage micrometer readings. Student's *t*-test was used for determinations of statistical significance.

Equilibration procedure. After mounting, the muscle was mechanically equilibrated at its *in situ* length in oxygenated artificial sea water for approximately 45 min followed by artificial sea water containing 2.5×10^{-5} M 5-HT for about 15 min. The muscle length was thereafter adjusted so that a small resting tension (approximately 1 mN) was obtained. This length was used as a reference length for further measurements (see below). The muscle was stimulated by 50 Hz alternating current at this length, with a stimulation period of 5 s and a stimulus interval of 5 min until consistent tension responses were obtained.

RESULTS

A. Force depression after active shortening

The effect of active shortening on the force producing capability in the ABRM of *Mytilus edulis* was investigated using a technique similar to that described previously (Edman 1975). Steps of different amplitude were applied during isometric phasic contraction. The steps used in this series of experiments all reached the same final muscle length, here designated L_1 . This length amounted to 90% of the muscle length at which a small resting tension (approximately 1 mN) was measured in artificial sea water containing 2.5×10^{-5} M 5-HT. The smallest release step, used as a control, had an

amplitude of $0.06 L_1$. The larger releases, test steps I and II, were twice and three times the size of the control step, respectively. The small control release, initiated when approximately half the maximum isometric tension value had been reached, caused a drop in tension to zero followed by an immediate redevelopement of force, while the larger, test steps allowed the muscle to shorten

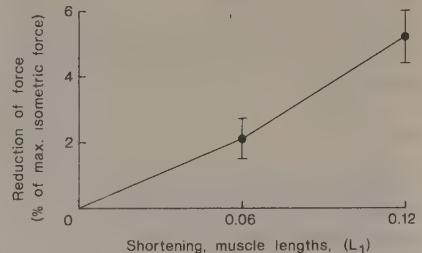


Fig. 2. Depressant effect of different amounts of active shortening during phasic contraction in the ABRM. Experimental procedure as described in Fig. 1. Ordinate: shortening induced depression, given as the difference in redeveloped force after the control and the test release steps expressed in per cent of unreleased isometric force at L_1 . Abscissa: muscle shortening in excess of control release. Mean values of 13 muscles. Vertical bars: S.E.

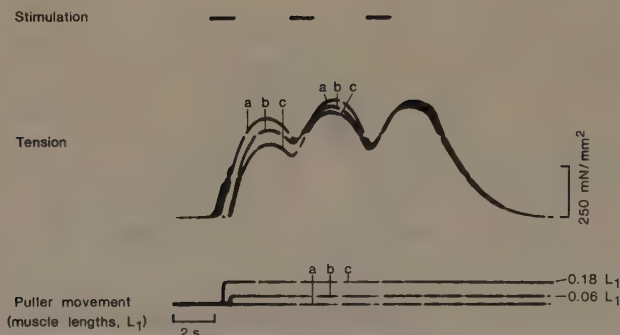


Fig. 3. Superimposed oscilloscope records of partially fused phasic contractions illustrating reversibility and duration of the shortening effect. Myogram a: unreleased isometric contraction. Myograms b and c: release steps produced during first contraction cycle. Note that the depressant effect of shortening, i.e. the difference in peak redeveloped force after test (0.18 L_1) and control (0.06 L_1) releases, decreased during continued isometric activity and had disappeared after approximately 8.5 s.

over an additional distance before starting to redevelop force. These larger steps were timed so that the tension redevelopment was initiated at the same moment after both the test and the control release steps. With the protocol used the control step and the two test steps were initiated and completed at muscle lengths within the plateau of the length-tension relation for this muscle (Cornelius & Lowy 1978). Since all release steps used, including the control step, were large enough to cause a complete drop in tension and since the effect of active shortening induced by the test releases were related to that obtained after the control release (see further below), any influence of the release per se to zero tension on the mechanical behaviour of the muscle was eliminated.

It can be seen in Fig. 1 that active shortening caused a slight reduction of the redeveloped force

and that this depressant effect was related to the amplitude of the release step. Fig. 2 summarizes data obtained in 13 different muscles. The depressant effect of active shortening has been expressed as the difference in redeveloped force after control release (0.06 L_1) and test release step, 0.12 L_1 and 0.18 L_1 , respectively, in per cent of isometric force. The shortening effect amounted to $2.1 \pm 0.6\%$ and $5.2 \pm 0.8\%$ (mean $\pm$ SE) after test step I and II, respectively. In Table 1 the depressant effect is presented as peak redeveloped force after the control and the two test releases used, test step I and II, respectively, in per cent of isometric force at L_1 . As can be observed the control step caused a reduction in force that was only $1.4 \pm 0.4\%$ (mean $\pm$ SE) of the unreleased isometric contraction response.

Table 1. *Depressant effect of active shortening during phasic contraction in the ABRM*

Results presented as mean $\pm$ SE, $n=13$

Shortening, muscle lengths, L_1	Peak redeveloped force in per cent of isometric force at L_1
0.06 L_1 , control step	98.6 ± 0.4
0.12 L_1 , test step I	96.4 ± 0.8
0.18 L_1 , test step II	93.1 ± 0.9

B. Reversibility and duration of the depressant effect of active shortening

Fig. 3 illustrates an experiment performed in order to study the reversibility and to determine the duration of the shortening effect. The muscle was stimulated for 1.3 s at intervals of 2.6 s. This procedure did not allow the muscle to relax completely after each stimulation and the contractions were therefore partially fused. As can be seen in Fig. 3 the depressant effect of active shortening was entirely reversible under these conditions. The more pro-

nounced force depression obtained after the test release step (compared to the depressant effect observed after the small, control step) disappeared and the same tension level was eventually reached after the control and test releases. This tension level was very nearly the same as that attained during the unreleased isometric contraction. The duration of the depressant effect, estimated from the onset of redevelopment of tension after shortening to the moment when the difference in force depression between test and control had disappeared, was found to be approximately 8.5 s. Four additional experiments confirmed that the depressant effect of active shortening was fully reversible and had completely disappeared after approximately 8–9 s.

C. The influence of the degree of activation of the contractile system on the depressant effect of active shortening

A series of experiments was carried out to examine if the shortening effect in the ABRM was influenced by the degree of activation of the muscle, i.e. by the amount of activator calcium in the myofibrillar space when the shortening occurs. The same release protocol was used as described in section A. As a means of varying the degree of activation the stimulation period was reduced in the same preparation from 8 s (giving maximum phasic contractile response) to 1.5 s which lowered the force output to approximately 50% of maximum phasic isometric force. The latter situation presumably represents a lower concentration of myofibrillar calcium (Kometani & Sugi 1978). The results are presented in Table 2 in which the shortening effect has been given as the difference in redeveloped force after the test and the control release steps expressed in per cent of the control (i.e. redeveloped force after the small shortening). As can be observed the shortening induced depression tended to be more pronounced when the releases were performed at the lower force output.

D. The influence of caffeine and dantrolene on the depressant effect of active shortening

It was of interest to find out if substances known to influence the state of activation in vertebrate skeletal muscle, such as caffeine and dantrolene, (e.g. Sandow 1965, Ebashi 1976, Van Winkle 1976, Mor-

Table 2. Comparison between depressant effects of active shortening during phasic contractions induced by 1.5 s and 8.0 s stimulation periods, respectively

No. of expt	Amplitude of shortening in excess of control release (muscle lengths, L_1)	Depression of peak force (% control)	
		Stimulation period	
		1.5 s	8.0 s
1	0.06	1.5	0.6
2	0.09	7.9	7.4
3	0.12	13.2	8.2
4	0.12	15.6	5.8
5	0.12	21.1	7.4

gan & Bryant 1977) had an effect on active shortening in the ABRM.

It has been shown that caffeine, in a concentration of 0.5 mM, has no appreciable effect on contractures induced by acetylcholine or by high $[K]_0$ in the ABRM (Sugi & Suzuki 1978). There is evidence that the mode of activation of the contractile system is different in the two cases. Thus, an inward movement of extracellular calcium is presumed to be the main cause of potassium-induced contractures, while acetylcholine-induced contractures are mainly associated with a release of intracellularly stored calcium (Sugi & Yamaguchi 1976). The ineffectiveness of caffeine on acetylcholine-contractures as well as on K-contractures has therefore been interpreted to mean that caffeine has no influence on either the inward movement of extracellular calcium or on the intracellular structures from which acetylcholine is supposed to release calcium (Sugi & Suzuki 1978). On the other hand, it has been demonstrated that caffeine in high concentrations (10 mM) can produce contracture tension, probably due to calcium release from the sarcoplasmic reticulum (e.g. Twarog & Muneoka 1972). In the present investigation caffeine caused an increase of the isometric phasic contraction. Thus, 0.12–0.25 mM caffeine increased the contraction response by about 10% of the value obtained in ordinary artificial sea water and this enhancing effect was approximately doubled when the caffeine concentration was raised to 0.50 mM. Fig. 4 shows the effect of active shortening in the presence of 0.12–0.50 mM caffeine. The shortening effect has here been expressed as peak redeveloped force after the test release (0.18 L_1) in per cent of

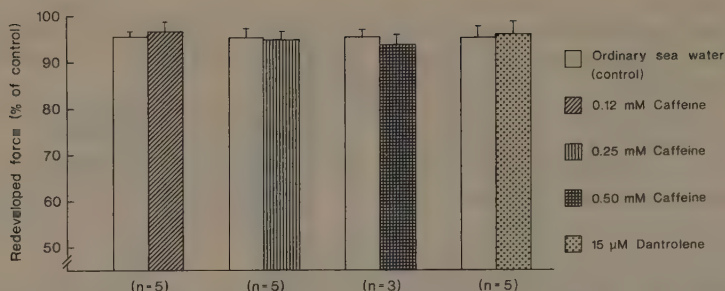


Fig. 4. The effect of active shortening in the presence of 0.12 mM caffeine, 0.25 mM caffeine, 0.50 mM caffeine and 15 µM dantrolene sodium, respectively, compared to the effect obtained in ordinary artificial sea water (paired observations). The redeveloped force after the test release step, 0.18 L₁, is expressed in per cent of peak redeveloped force after the control step (0.06 L₁). The shortening induced depression was not significantly altered in the presence of caffeine and dantrolene sodium (Mean ± SD).

the control (i.e. the force redeveloped after the small shortening, 0.06 L₁). As is illustrated in Fig. 4 the presence of caffeine, 0.12 mM to 0.50 mM, did not significantly alter the effect of active shortening. At none of the three different caffeine concentrations used the depressant effect was found to be statistically different from the value obtained in plain sea water ($p > 0.05$, paired observations).

Dantrolene reduces the twitch tension value in vertebrate skeletal muscle, likely due to an inhibition of calcium release in response to membrane excitation (Van Winkle 1976, Desmedt & Hainaut 1977, Morgan & Bryant 1977). The contractile activity in mammalian cardiac and smooth muscle, on the other hand, is reported to be essentially unaffected by dantrolene (Butterfield & Ellis 1973). In the anterior byssus retractor muscle of *Mytilus edulis* 15 µM dantrolene sodium was found to increase the isometric tension response by approximately 10%. As can be observed in Fig. 4 the redeveloped force after active shortening (in per cent of the control) amounted to $95.1 \pm 2.3\%$ and $95.8 \pm 2.7\%$ (mean ± SD) in the ordinary solution and in the presence of 15 µM dantrolene, respectively, i.e. dantrolene did not significantly affect the shortening induced depression ($p > 0.60$, paired observations).

E. The influence of varied tonicity of the extracellular medium on the depressant effect of active shortening

The depressant effect of active shortening was studied while the muscle was immersed in artificial

sea water of varied tonicity ranging between 0.9 T and 1.2 T of the normotonic (T) solution. The results from these experiments are summarized in Fig. 5.

A hypertonic solution reduced the isometric phasic contraction response by approximately 30% of the isometric tension value obtained in the normotonic medium. This decrease in mechanical response is likely to be mainly attributable to osmotic shrinkage of the preparation rather than to increased ionic strength in the myoplasm, since this has been presumed to explain the reduction of acetylcholine- and potassium-induced contractures observed in sucrose-hypertonic solutions (Sugi et al. 1977). This proposition has been supported by the finding that altered ionic strength (range 0.07–0.28 M) in the skinned fibre preparation of the ABRM has only a slight influence on maximum developed tension (Cornelius 1980). As is evident from the values presented in Fig. 5 the depressant effect of active shortening, calculated as described in section D, was not appreciably altered by increasing the tonicity of the extracellular medium. The redeveloped force in normotonic solution was $95.4 \pm 2.0\%$ while the corresponding value obtained in the hypertonic solution was estimated to be $94.2 \pm 3.8\%$ (mean ± SD). This difference was not statistically significant ($p > 0.30$, paired observations).

A decrease in tonicity to 0.9 T (by reduction of the NaCl concentration from 200 mM to 165 mM) did not markedly affect the isometric phasic con-

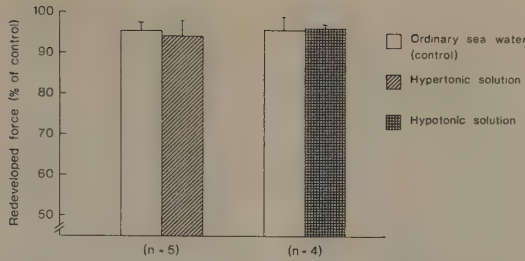


Fig. 5. The effect of active shortening at varied tonicity of the extracellular medium. The force depressant effect, calculated as described in Fig. 4, was not significantly affected by the hypertonic and hypotonic solutions (Mean $\pm$ SD).

tracture tension. Nor was there any detectable influence of hypotonicity on the depressant effect of shortening as is illustrated in Fig. 5. The redeveloped force in ordinary sea water and in hypotonic solution amounted to $95.6 \pm 3.2\%$ and $96.2 \pm 1.0\%$ (mean $\pm$ SD), respectively. As might be expected this slight difference was not proven to be statistically significant ($p > 0.70$, paired observations).

DISCUSSION

Active shortening of the ABRM of *Mytilus edulis* results in a transitory reduction of the contractile activity. This shortening induced depression is likely to represent the same phenomenon as previously observed in vertebrate striated muscle in response to filament sliding (Edman 1975, 1980). The depressant effect has been shown to be related to the amplitude of shortening, but to be independent, over a wide range, of the speed of shortening and the force output during the shortening phase (Edman 1975). Results obtained in intact frog muscle fibres suggest that the shortening effect is not based on altered kinetics of release and reuptake of activator calcium in the excitation-contraction coupling (Edman 1975). This view is further supported by the demonstration that a transitory depressant effect of shortening also occurs, in a very similar way, in frog skinned muscle fibres activated in a calcium-buffered medium (Ekelund & Edman 1982). The evidence obtained in experiments on vertebrate striated muscle suggests that active shortening has an effect on the myofilament system itself probably leading to reduced binding of activa-

tor calcium to the regulatory troponin-tropomyosin complex (Edman 1980, Ekelund & Edman 1982).

Previous studies of the depressant effect of active shortening were performed on muscles that are all considered to be regulated by the troponin-tropomyosin complex. It is therefore of interest to note that a similar depressant effect, although quantitatively smaller, occurs in the ABRM in which the actin-linked regulatory system is regarded to be of minor importance. In this mollusc muscle the myosin regulatory light chains are considered to inhibit myosin interaction with actin in the relaxed muscle. Contraction is thought to be induced by binding of activator calcium to specific sites on the myosin; this would relieve the inhibitory effect and allow interaction between the actin- and myosin filaments (Kendrick-Jones et al. 1970, Szent-Györgyi et al. 1973, Lehman & Szent-Györgyi 1975, for review see Kendrick-Jones & Scholey 1981). However, enzymatic and electrophoretic studies have recently indicated the presence of troponin-like proteins in several molluscan muscles (Goldberg & Lehman 1978, Lehman 1981) but it is still unclear to what extent an actin-linked Ca^{2+} -regulatory system contributes to the control of the mechanical activity in these muscles. Thus, the possibility exists that the ABRM of *Mytilus edulis* has a dual Ca^{2+} -regulation, mediated by both the actin-linked troponin-tropomyosin complex and the myosin and associated light chains.

An important aspect of the shortening induced depression is that it is completely reversible (Edman 1975). This feature also characterizes the depressant effect observed in the ABRM of *Mytilus*

edulis. This effect should therefore be separated from the active tension loss that occurs in the ABRM above and near L_0 (defined as that muscle length at which a small resting tension, approximately 1 mN, can be detected in the presence of 5-HT at a concentration of 2.5×10^{-5} M) when the muscle has been stimulated with acetylcholine at a longer length and thereafter *passively* shortened (Cornelius & Lowy 1978). The shortening effect described in the present investigation is also distinct from that observed in mammalian smooth muscle. In this preparation it has been reported that active shortening from muscle lengths greater than L_0 (defined as that muscle length at which passive tension first appears) results in an irreversible loss of contractility. This loss of contractile strength was found to be cumulative with repeated contractions from lengths greater than L_0 and to persist at all muscle lengths, including lengths shorter than L_0 (Peterson & Paul 1974).

There are, theoretically, several different mechanisms that could underlie the force depressant effect that is induced by active shortening in the anterior byssus retractor muscle of *Mytilus edulis*. The following possibilities have been considered for the shortening induced depression 1. Active shortening might lead to non-uniformity in length of the contractile units. 2. The kinetics of the release and reuptake of activator calcium are altered by shortening. 3. A structural change in the myofilament system itself that leads to a decreased response to activator calcium. It is reasonable to exclude a non-uniform distribution in length of the contractile units ('sarcomere length') as a mechanism underlying active shortening. The depressant effect is entirely reversible and it is reasonable to assume that such a non-uniformity in the overlap between the A- and I-filaments would be maintained or become even more pronounced as the contractile activity proceeds. Furthermore, the finding of a plateau with relatively distinct corners on the active length-tension curve in the ABRM suggests an ordered filament arrangement in the muscle and this plateau persists after quick release of the muscle (Cornelius & Lowy 1978). The long lasting nature of the depressant effect of shortening, 8 to 9 s, and the fact that the force depression does not disappear merely by restimulation of the muscle make it unlikely that the effect is due to a reduced release of activator calcium. It is also unlikely that active shortening would enhance the

reuptake of activator calcium. In such a case the total duration of the contractile response would diminish but as is evident from Fig. 1 this does not occur. A change in the myofilament system itself caused by active sliding of the filaments and resulting in a reduced degree of interaction between the actin- and myosinfilaments therefore remains as the most conceivable interpretation.

The depressant effect of active shortening in the ABRM is considerably smaller than the force depression observed after active shortening during a twitch in vertebrate skeletal muscle (Edman 1975). In ABRM shortening of 0.06 L_1 and 0.12 L_1 (in excess of control) resulted in a force depression amounting to 2.2% and 5.6%, respectively, of the control (i.e. of the force redeveloped after the control step, 0.06 L_1). This is to be compared with a depressant effect of 35–40% and 55–60%, respectively, during a twitch of a frog single muscle fibre, after release steps of equivalent amplitude (Fig. 2 in Edman 1975). It should be noted that the magnitude of the depressant effect of shortening is critically dependent on the amount of activator calcium (see further below). Optimal phasic contraction of ABRM presumably reflects partial activation since application of acetylcholine was shown to generate an approximately 60% greater tension response, which was suggested to be due to a more complete activation (Cornelius & Lowy 1978). Furthermore, even the shortening induced depression during sub-optimal *phasic* contraction was smaller (Table 2) than that obtained during a twitch. It therefore seems reasonable to conclude that the depressant effect of active shortening is less pronounced in ABRM than in vertebrate striated muscle.

Thus, in the ABRM the shortening induced depression can be considered to be quantitatively smaller than in vertebrate striated muscle. However, certain qualitative similarities between the depressant effect of active shortening in this mollusc muscle and in vertebrate striated muscle exist, indicating that the underlying mechanism is the same in both cases.

In vertebrate striated muscle, the effect of active shortening is critically dependent on the degree of activation of the muscle fibre when the motion occurs. The depressant effect is much more pronounced if shortening is performed during a single twitch and during a partially fused tetanus than during a fused tetanus. Furthermore, the shortening effect is very much reduced during a twitch and is

virtually abolished during a tetanus after addition of caffeine (Edman 1976, 1980), a substance that presumably increases the amount of activator calcium around the myofilaments in response to excitation of the fibre membrane (e.g. Sandow 1965, Ebashi 1976). Finally, the significance of the calcium concentration in the myofibrillar space has been established in frog skinned fibre preparations, where the free calcium concentration can be carefully controlled and kept constant (Ekelund & Edman 1982). These studies demonstrate that there is a progressive reduction of the depressant effect of shortening as the calcium ion concentration is raised. The present investigation shows that also in the ABRM the depressant effect of active shortening is dependent on the degree of activation of the muscle. Active shortening performed at reduced isometric force resulted in an increase of the depressant effect (see Table 2). The addition of caffeine caused enhancement of the isometric contraction response in the ABRM, presumably reflecting an increased amount of activator calcium in the myofibrillar space. This additional amount of calcium around the myofilaments did not significantly affect the already slight movement effect in this mollusc muscle. This observation is in accordance with results obtained in vertebrate, striated muscle, where the influence of caffeine is less pronounced when the shortening induced depression is originally quite small, as in response to active shortening performed during the plateau of a tetanus.

The tonicity of the extracellular medium influences the depressant effect of active shortening in vertebrate skeletal muscle (Ekelund unpublished). In a solution made hypotonic by reduction of NaCl the effect of active shortening in isolated intact skeletal muscle fibres of the frog was increased while the reversed effect was obtained in a solution made hypertonic by addition of sucrose. This might be due to changes in fibre width. However, alteration of the intracellular ionic milieu is also of importance, since an increase in ionic strength, from 105 to 235 mM, was found to reduce the movement effect in vertebrate skinned fibre preparations (Ekelund & Edman 1982). The small depressant effect observed during a fused tetanus was much less affected than the larger shortening effect obtained during a single twitch (Ekelund unpublished). It is therefore of interest to note that the relatively small depressant effect of shortening observed in the mollusc muscle was not markedly

sensitive to a change in the tonicity of the extracellular medium.

A decrease in the calcium binding to troponin resulting in a reduced degree of interaction between the A- and I-filaments has been proposed as a plausible explanation of the depressant effect of active shortening in vertebrate striated muscle (Edman 1980, Ekelund & Edman 1982). The Ca^{2+} -regulatory system of the ABRM of *Mytilus edulis* has long been considered to be essentially myosin-linked. However, as already pointed out, the coexistence of an actin-linked troponin-tropomyosin regulatory system cannot be ruled out. Indeed, both actin- and myosin-linked Ca^{2+} -regulatory systems have been indicated in mollusc muscles (for references, see above). An attractive hypothesis would be that the depressant effect of active shortening in the ABRM is effectuated by a change of the actin-linked Ca^{2+} -regulatory complex. Such a hypothesis may explain the qualitative similarities between mollusc and vertebrate skeletal muscles. The finding that the depressant effect observed in the ABRM is quantitatively smaller than in vertebrate striated muscle is understandable if the troponin-tropomyosin system is of minor importance. However, it cannot be excluded that the shortening induced depression in the ABRM is due to an effect upon the myosin-linked Ca^{2+} -regulatory system. This would mean that the myosin-linked mechanism is considerably less sensitive to active shortening than the regulatory troponin-tropomyosin complex in vertebrate skeletal muscle.

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